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MIT/LCS/TM-445.b

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(revision of TM 445)

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May 1991

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An Autoimmune Mechanism for AIDS' T4 Lymphopenia

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May 21, 1991

Abstract.

We put forward a new model for the T4 lymphopenia occurring in AIDS. In essence, we present a mechanism whose net effect is blocking the generation of T4 cells during HIV infection. Supporting evidence for our mechanism is derived from experiments in the recent literature.

The etiological agent of AIDS has been identified in the HIV virus. 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.

There still is, however, a mysterious aspect to this illness.

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, many AIDS researchers (including Gallo and Montagnier [1]) 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 [2]). 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 [3], 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.* 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 peripheral 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. Indeed, the experiments of Kruisbeek, Mond, Fawlkes, Carmen, Bridges, and Longo [6] show that, in mice, the absence of I-a-bearing antigen presenting cells in the thymus (obtained by neonatal treatment with anti-I-a) causes the absence of T cells of the Lyt-2^- ,L3T4^+ lineage. Thymic MHC-II molecules need not to be absent, however, for the purpose of inhibiting the maturation of T4 cells. It may be enough to block them by non-depleting antibodies binding to, and thus inhibiting, the site(s) involved in T4-cell differentiation. This blockage may effectively 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:

> As for all foreign organisms, our body must react to HIV by 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 (a group of) antibodies raised against HIV must have a binding affinity *very close* to the one of CD4. Let's call *CD4like* such an antibody, or group of antibodies. Like the copy of a key, *CD4like* 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 mechanism. The release into the blood of *CD4like* caused by HIV will not be a one-time affair. HIV has a very long latency, during which it seems to be "invisible" to the immune system. In these conditions *any* virus would remain present for a long time. Assume, in fact, that some virus *V* manages to infect many cells of the organisms before any defense against it can be mounted. At this point, each infected cell has a separate, individual destiny. Thus, within the infected cells, the proper conditions for *V* to become active will occur at *random* and *independent* times. If the expected life time of the infected cells and the expected latency of virus *V* both are --say-- several years, the result of the initial infection is that *V* (or its proteins) will reappear at random times for several years. If the cells initially infected are sufficiently many, these "random, reappearance times" will be frequent enough for *V* (or its products) to be essentially continually present and detectable in the organism 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 virus *V* whenever it becomes visible.) Thus HIV "continual reappearance," continually elicits powerful secondary responses of the immune system. Consequently with our hypothesis, (different) plasma cells will continually produce (different) antibodies against HIV, *including CD4like* ones. In fact, despite its constant mutability, HIV maintains its capability of binding CD4; thus, once a *CD4like* 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.[α]

An important, novel feature of our hypothesized mechanism is that it provides a better model for the 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 *CD4like*, and thus mislead the organism into producing fewer T4 cells.[#] These cells have a finite life time and must 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, AIDS patients might perhaps 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' [4] 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.

Pros and Cons

Binding sites. On the CD4 receptor, are the binding sites for MHC-II molecules and gp 120 overlapping or similar anyway? (If not, antibodies raised against gp 120 would be as unrelated to MHC as an antibody against another random epitope of CD4.) Evidence that these binding sites are overlapping is given by Clayton, Sieh, Pious, and Reinherz [8]. The residue of CD4 involved in gp 120 binding have been localized in the CDR2 region, and they show that CDR2 mutations of CD4 preventing gp 120 binding also prevent MHC-II binding. (On the other hand, the authors also provide evidence that the binding sites may not be totally identical; that is, that the MHC-II binding site properly contains the gp 120 one. In fact, they show that some mutations preventing MHC-II binding do not affect gp 120 binding.)

Thymic role. The thymus is essential, early in life, for developing an adequate T-cell repertoire; it does not, however, seem essential in adult or even young individuals. Indeed, during cardiac surgery, the thymus is often removed without any apparent consequences for the immune system; moreover, thymic output in adult individuals is a tiny trickle. How, then, can *CD4like* have any disruptive action? Our answer is that the thymus may still be useful later in life for replacing lost T cells, a task easily accomplishable by its reported tiny

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[α] It should be noticed that our hypothesis is distinct from the more traditional one of anti-idiotype networks involving an internal image of the antigen. The traditional scenario involves an initial production of an antibody, and then the production of an anti-antibody, carrying an internal image of the antigen. In the case of *CD4like*, there is no second antibody; instead, the first antibody produced against a foreign antigen resembles self --namely, CD4.

[#] Indeed, *CD4like* does not directly deplete T4 cells. Else, it should also deplete T4 cells, since they too express MHC-II molecules.

output. Moreover, surgeons may leave behind some thymic tissue; enough, in normal conditions, to guarantee T-cell replacement.

T-cell life span. In order for *CD4like* to be a significant co-factor in AIDS' T4 lymphopenia, T4 should not be as "eternal" as neurons. Is this true? Any estimate of the life span of T cells is quite controversial. Nonetheless, let's assume for a moment that the expected life time of a T4 cell is, say, 7 years and that AIDS' T4 lymphopenia is principally due to the action of *CD4like*. This assumption has a noticeable consequence: after seroconversion, the number of T4 cells in an infected individual should, roughly, decrease by the same, absolute amount each year. This can be argued as follows. First, in a mature, infected adult, the ages of T4 cells must be distributed almost uniformly. Second, we expect that in the first year essentially all (and only) the cells that, at the time of seroconversion, were six-year old will die (and will not be replaced), in the second the cells that were 5-year old, and so on. Indeed, this logical consequence appears to be roughly in agreement with what is actually measured in reality, and, except perhaps for an apparent "plateau," the decline of T4 cells looks linear.[$] If it were a pure coincidence, it would be a quite exceptional one. In fact, if the T4 lymphopenia solely depended on HIV's depleting action, the decline of T4 cells would be exponential rather than linear! With the progress of the infection, the number of HIV particles increases, thus the number of killed T4 cells per year should also increase --except, possibly, at the very end, when there would not be enough T4 cells left.[%]

Specificity of CD4like. It appears that sera from AIDS patients do not react with HIV-1 isolates unrelated to the one causing the patient's infection. If *CD4like* were present in appreciable amounts, should it not bind to all HIV-1 isolates? First, it should be noticed that the amount of *CD4like* in the serum of AIDS patients may not be too telling, since these antibodies are already binding. Our answer, however, is that we are facing another example of "canyon effect." The binding site for CD4 on gp 120 appears to be recessed, or hard to access, or clouded by sugar, or all of the above. This may make it very hard for the organism to produce antibodies capable of too successfully binding gp 120. For instance, binding may be easy for monovalent structures --like CD4-- but hard for a bivalent ones -- like all antibodies, including *CD4like*. As a result, *the antibodies successfully binding HIV would be recognizing only its more variable epitopes*. The high mutability of the virus may thus explain why it is so elusive within the same organism, and why no reaction to unrelated HIV isolates is noticed in the sera of AIDS patients --independently of whether an appreciable amount of essentially identical, *CD4like* antibodies would be present. Indeed, in light of the fact that the CD4-binding site is highly preserved, if successful antibodies against it were produced, the immune system could also successfully fight HIV. It should also be noticed, though, that while the relative inaccessibility of the crucial site of gp 120 may prevent antibody binding, it needs not to prevent the manufacturing of antibodies.

[$] I am not arguing here that this linearity proves that the life time of T4 cells is exactly 7 years; it may actually be more: a moderate depleting action of HIV may in fact accelerate a bit the rate of their disappearance, while substantially preserving the linearity of the process.

[%] Here, admittedly, many a simplifying assumptions are made --such as that mature cells do not proliferate, etc. Moreover, linear effects may be the net result of several, opposite, trends, each of which is non-linear. For example, the killing rate of HIV may be exponential, but is compensated by newly produced T4 cells. In any such a case, however, the "too-few-infected-cells" phenomenon which we are trying to explain would become even more mysterious. In fact, infected T4 cells would be even fewer than expected.

Indeed, the relevant binding site of gp 120 may become more accessible once gp 120 has been "digested" by an antigen presenting cell.

On the whole, while one can express reasonable reservations, our hypothesis cannot be refused on the basis of known facts, and some experimental verification should be sought.

Testing The Mechanism

Ideally, one would like to develop an essay for *CD4like* antibodies and then determine whether there is a correlation between the quantity of *CD4like* present and the decline of T4 cells. More indirect tests, however, may also be useful.

Such an indirect test may consist of monitoring T-cell reconstitution of irradiated animals, both in the presence and in the absence of soluble CD4. Radiation, causing a sudden drop in T-cell level, may allow one to conduct a shorter experiment. Working with soluble CD4 avoids isolating *CD4like* among many candidate antibodies, and a new successful method for producing soluble rat CD4 has recently been obtained by Davis, Ward, Puklavec, Willis, Williams, and Barkley [5].&

Thymus cultures and bone-marrow cells may be used to verify that the thymic tissue of AIDS patients indeed produces fewer T4 cells than normal. Of course, an effective method must be used so to trace only newly generated T cells.

An elegant test has been suggested by Herman Eisen [7] for determining whether the non-polymorphic regions of thymic MHC-II molecules of AIDS patients have some proteins bound to it, thus providing an indirect evidence for the existence of *CD4like*. The test consists of showing that some (fluorescent) antibodies for non-polymorphic epitopes of MHC-II molecules fail to bind the thymic cells of AIDS patients.@ The same experiment can be conducted using B cells rather than thymic cells, since they too express MHC-II molecules.

In Sum

The CD4like mechanism explains some puzzling phenomena occurring in AIDS: why the infected T4 cells are so few, why T4 cell loss starts only after antibody formation against HIV, while a potent immune response --differently than for most viruses-- is ineffective in the HIV case, why T4-cell decline is almost linear, and so on. At the same time, it may warn us about possible side effects of some vaccines.

It should be noticed, however, that above we have described just the most plausible way, in the light of established biological mechanisms, for *CD4like* to influence the level of T4

& It is, however, important to notice that, due to the difference in molecular weight and structure between *CD4like* and soluble CD4, the risk exists that only the former protein may successfully block thymic MHC-II cells. Moreover, soluble CD4 would last in circulation much less than an antibody, and a viable method must be found to keep high level of it in the blood.

@ A more refined version of the test consists of using both antibodies (labelled "green") for the polymorphic regions of MHC-II proteins, and (labelled "red") for the non-polymorphic regions, and then studying the green/red binding ratio for B cells of AIDS patients and healthy individuals.

cells. So little, though, is known about T-cell regulation, that many subtler possibilities exist for *CD4like* to affect the level of 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,-- since this is usually well correlated to the total amount of T4 cells. Thus *CD4like* may even mislead the organism into believing that there are many more T4 cells than actually present and trigger a depleting, regulatory mechanism. As for another example, an anti-antibody may be raised against *CD4like* capable of binding CD4 and depleting T4 cells.) For this reason let us summarize our autoimmune model for AIDS' T4 lymphopenia in a more open-ended manner. Namely,

CD4like 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 the Experiment.

Acknowledgements

I wish to wholeheartedly thank Leonard Adleman for introducing me 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 very grateful thanks also to David Raulet for his wonderful critique of an earlier version of this paper . Section "Pros and Cons" essentially consists of our debate.

Thank you, finally, Cecil (Green) for supporting my investigating a new field. I am enjoying it tremendously.

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