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Bioessays. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Bioessays. 2019 January ; 41(1): e1800128. doi:10.1002/bies.201800128.

HIV disease progression: Overexpression of the ectoenzyme CD38 as a contributory factor?

J. C. Rodríguez-Alba[1], **A. Abrego-Peredo**[2], **C. Gallardo-Hernández**[2], **J. Pérez-Lara**[2], **J. W. Santiago-Cruz**[3], **J., W. Jiang**[4], and **E. Espinosa**[5,*]

[1]Flow Cytometry Core Facility, Instituto de Ciencias de la Salud, Universidad Veracruzana, Xalapa, Mexico

[2]Doctorado en Ciencias de la Salud, Instituto de Ciencias de la Salud, Universidad Veracruzana, Xalapa, Mexico

[3]Maestría en Ciencias de la Salud, Instituto de Ciencias de la Salud, Universidad Veracruzana, Xalapa, Mexico

[4]Department of Microbiology and Immunology, and Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, Charleston, SC, USA, 29425

[5]Laboratory of Integrative Immunology, National Institute of Respiratory Diseases (INER), Mexico City, Mexico.

Summary

Despite abundant evidence associating CD38 overexpression and CD4 T cell depletion in HIV infection, no causal relation has been investigated. To address this issue, we propose a series of mechanisms, supported by evidence from different fields, by which CD38 overexpression could facilitate CD4 T cell depletion in HIV infection. According to our model, increased catalytic activity of CD38 may reduce CD4 T cells' cytoplasmic nicotinamide adenine dinucleotide (NAD), leading to a chronic Warburg effect. This would reduce mitochondrial function. Simultaneously, CD38's catalytic products ADPR and cADPR may be transported to the cytoplasm, where they can activate calcium channels and increase cytoplasmic Ca^{2+} concentrations, further altering mitochondrial integrity. These mechanisms would decrease the viability and regenerative capacity of CD4 T cells. These hypotheses can be tested experimentally, and might reveal novel therapeutic targets.

Graphical Abstract

In HIV disease, CD38 expression and its catalytic activity are increased in several cell types. This may lower intracelular NAD levels in CD4 T cells, shifting their metabolism and compromising mitocondria. CD38's products may increase cytoplasmic Ca^{2+}. These effects may reduce CD4 T cell viability and regeneration capacity.

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[*]**Corresponding author:** Enrique Espinosa, espinosa@iner.gob.mx.

Conflict of interests statement

Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas has made an application for a patent based on the ideas presented this manuscript, with EE as inventor (Instituto Mexicano de la Propiedad Industrial, September 26, 2018, file MX/a/2018/011698, application number MX/E/2018/072036).

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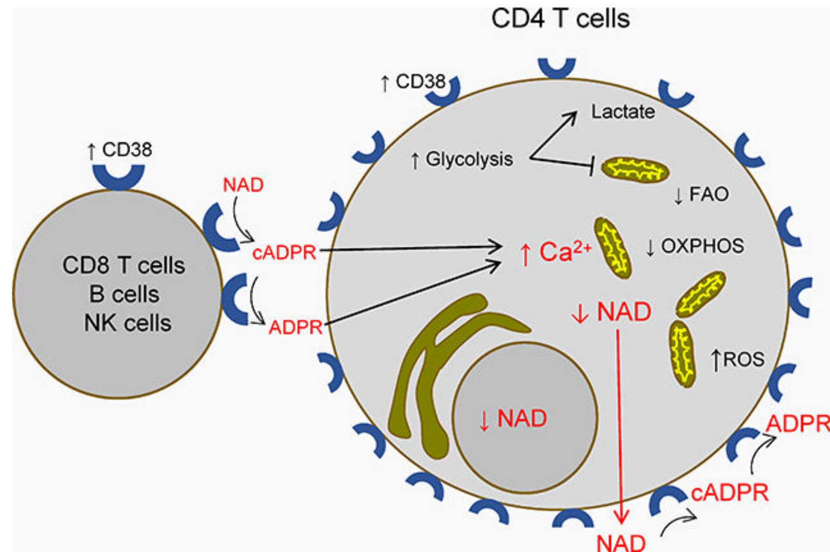

Keywords

HIV/AIDS; CD38; immune activation; CD4-Positive T lymphocytes; NAD; Immunometabolism; Warburg effect

1. Introduction

1. 1 CD38 and Immune activation in HIV infection

An early finding in blood from patients with Acquired Immune Deficiency Syndrome was the presence of a large number of lymphocytes expressing the surface protein CD38.[1–3] Because CD38 is expressed by activated T cells, extensive research on immune activation in HIV infection ensued. Extensive evidence shows a strong correlation between the proportion of activated circulating T cells and HIV disease progression, as measured by survival time,[4,5] time to onset of opportunistic infections,[6,7] and loss of circulating CD4 T cells.[8–11] Under antiretroviral treatment, T cell activation is associated with the immune reconstitution inflammatory syndrome (IRIS)[12,13] and with insufficient recovery of CD4 T cells.[14–16] In this research area, however, CD38 has been measured as a mere activation marker,[17] leaving unanswered the question of whether CD38 overexpression has a causal relation with these outcomes.

1. 2 CD38 is a strong and independent predictor of CD4 T cell depletion

Looking back at early studies, one can see that CD38 overexpression is a very salient aspect of chronic activation in HIV infection. The number of CD38 molecules on the surface of CD8 T cells (as well as the proportion of CD8 and CD4 T cells expressing CD38) add capacity to predict the onset of AIDS to blood counts of CD4 T cells, HIV's main target.[9,18,19] CD38 density on the surface of CD8 T cells could predict HIV disease progression and death, and was found to be independent of blood viral load (number of HIV copies per mL of blood).[20] CD38 expression on CD8 T cells was consistently predictive throughout the whole duration of untreated HIV infection, while viral load lost its predictive capacity in

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the long term, and CD4 T cell count had a low predictive capacity in early infection.[21] Importantly, CD38 expression on CD8 T cells was a stronger predictor than several other activation markers, including soluble mediators of inflammation, and the percentage of T cells with different combinations of HLADR and CD38 expression, as indicators of immune activation.[5] More direct indicators of T cell activation, like proliferation and cell cycle, also predict HIV disease progression.[22–24] In this regard, we have found that CD38 expression on T cells from patients with HIV correlates with lower circulating CD4 T cell counts independently of the frequency of cycling cells,[25] which further describes CD38 independence as predictor.

1.3 CD38 as predictor: implications for HIV disease pathogenesis

A possible implication of CD38's statistical independence is that CD38 is reflecting a pathogenic mechanism independent of killing of CD4 T cells by infecting virions. An alternative implication is that CD38 itself is facilitating CD4 T cell loss. Such role requires the involvement of a particular biological function of CD38. At the same time, CD38 overexpression should increase its biological activity, which should be present in the different body compartments of CD4 T cells in the setting of HIV infection. This increased function should promote CD4 T cell loss or decreased cell renewal of CD4 T cells (which are already decimated by HIV and keep being eliminated by the cytopathic effect of cellular infection by virions). Currently, there are findings from different fields supporting that these conditions may concur in HIV infection. This scenario poses a number of hypotheses. Throughout this text we will provide an account of mechanisms supporting these hypotheses, and how they can be addressed experimentally.

2. Problem and proposed approach

It is currently unknown whether CD38 actually participates as an indirect pathogenic mechanism of HIV disease. We propose that CD38 overexpression may promote CD4 T cell depletion in the setting of HIV infection via its enzymatic functions. This would add to HIV's direct effects on CD4 T cells. The mechanisms that can mediate this indirect pathogenic role can be compiled in the following hypotheses.

2.1 In HIV infection, excessive chronic CD38 expression by different cell types causes an increase in CD38's catalytic activity in different body compartments.

2.2 CD38's catalytic activity can decrease intracellular concentrations of nicotinamide-adenine dinucleotide (NAD) in CD4 T cells.

2.3 CD38 catalysis yields cADPR and ADPR, which are transported to the cytoplasm.

2.4 A decrease in cytoplasmic NAD may promote a metabolic switch favoring glycolysis over mitochondrial respiration (Warburg effect).

2.5 cADPR and ADPR, when transported into the cell, can increase cytoplasmic Ca^{2+} concentration.

2.6 Ca^{2+} increase, in addition to NAD depletion, may affect mitochondrial integrity.

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2.7 These mechanisms can result in a reduced capacity survive and proliferate of CD4 T cells from HIV^{+} persons.

These hypotheses, integrating our model (Figure 1), are based in current knowledge of CD38 as an enzyme (see next) and can be addressed experimentally (Table 1).

3. Evidence supporting CD38-mediated pathogenesis in HIV disease

3.1 Biological functions of CD38

3. 1. 1 CD38 enzymatic activity—The first evidence of CD38 expression on human leukocytes was obtained employing the monoclonal antibody OKT-10.[26] Thereafter, it was reported that CD38 is expressed by activated human T cells and several cancer cell types. CD38 shows a substantial similarity with an enzyme from *Aplysia californica* that catalyzes the conversion of nicotinamide adenine dinucleotide (NAD) to cyclic adenosine diphosphate ribose (cADPR), and the hydrolysis of cADPR into adenosine diphosphate ribose (ADPR).[27] Human CD38 gene is encoded at chromosome 4 p15.32.[28] The protein is formed by 300 amino acids, and has a molecular mass of 42–45 KDa.[29] CD38 is a type II transmembrane glycoprotein with a 21-amino acid cytoplasmic N-terminal domain, a 21-amino acid transmembrane domain, and a 258-amino acid extracellular catalytic and receptor domain.[30] CD38 belongs to the ribosyl family of NAD/ADP cyclases. Its extracellular portion possesses a catalytic pocket buried in the middle cleft of the protein,[31] where amino acid positions critical for these catalytic activities are found.[32,33] Importantly, the catalytic portion of human CD38 has been mapped to its extracellular domain.[31]

In an acid medium, CD38 may also catalyze the synthesis of nicotinic acid-adenine dinucleotide phosphate (NAADP);[34] however, at neutral pH, CD38 actually hydrolyzes NAADP.[35] This constrains the sites where NAADP is produced,[36] rendering unlikely the synthesis of NAADP by CD38 oriented to the extracellular milieu.

3. 1. 2 CD38 as a possible receptor—A possible function of CD38 as a receptor has been suggested, partly based on the effect of cross-linking CD38 with specific monoclonal antibodies. For instance, antibody IB4, initiates T cells signaling within a subset of membrane rafts[37] and recruits the signal transduction molecules TCR/CD3ζ chain, ZAP-70, phospholipase C-γ, Raf-1/mitogen-activated protein kinase, and calcium mobilization.[38–40] Previous work has proposed that CD31 and hyaluronic acid may act as natural CD38 ligands; however, this was based mainly on the binding of these molecules to CD38[41,42] and on the induction of calcium fluxes, cytokine production, and gene expression on heterogeneous cell populations or in transformed cell lines.[41,43] Agonistic monoclonal antibodies do not necessarily represent a specific ligand, and existing studies using putative ligands cannot be extrapolated to primary T cells. Evidence of a receptor function of CD38 should include a well described ligand with a physiological role on defined T cell functions. Therefore, we focus on CD38's catalytic functions.

3.2 High CD38 activity in CD4 T cell-homing tissues in HIV infection

Our model implies that CD4 T cells are exposed to an increased CD38 catalytic activity. As mentioned above, CD4 and CD8 T cells from patients with HIV express CD38 more

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frequently than cells from uninfected controls, and CD38-expressing CD8 T cells from HIV$^+$ patients show more CD38 molecules on its surface than those of uninfected people.[18,19] Moreover, CD8 T cells are expanded in HIV infection,[44–46] which further increases the number of CD38 molecules in blood. In addition to T cells, a greater proportion of NK[47,48] and B cells[49] from HIV$^+$ persons express surface CD38, while patients' monocytes show a tendency to greater CD38 expression.[50] These cells can further increase the amount of CD38 in contact with blood and tissues. Given that CD38 expression dictates NAD concentration,[51,52] we can expect that increased CD38 activity of CD38 in blood yields a decrease in blood NAD levels, unless NAD removal is somehow compensated. This possibility can be investigated by measuring CD38 expression and the actual catalytic activity of CD4 T cells, CD8 T cells, and other cell populations from HIV$^+$ patients and healthy controls. Concurrently, blood NAD content can be analyzed (Table 1).

Given that CD38's activity is determined by its expression,[27,52,53] CD4 T cells of HIV-infected patients will be exposed to a milieu with an enhanced CD38 catalytic activity.[54] In HIV infection, CD38 expression is also increased in T cells from lymph nodes,[55–58] lymphoid tissue associated with the small[59] and large[60,61] intestine, and the female genital tract.[62] This would extend the span of increased CD38 activity to most of the homing sites of CD4 T cells. Given this relative ubiquity of CD38, it can be expected that its overexpression affects other tissues and cell types. In agreement with this, CD8 T cells suffer activation-associated exhaustion in HIV infection,[63] even though they are not infected by the virus. In several tissues, age-related increased CD38 expression causes a gradual decrease of NAD levels with aging,[52] which promotes insulin resistance, mitochondrial dysfunction, and senescence.[64] Interestingly, accelerated senescence is in fact observed in patients with HIV (reviewed elsewhere[65]). Therefore, it could be very informative to determine CD38's activity and NAD concentrations in blood and homing tissues of CD4 T cells, both in people with HIV infection, and healthy controls (Table 1).

Our model refers specifically to the setting of HIV infection. CD38 is normally expressed by several cells in lymphoid tissues, like CD8 T cells in colon-associated lymphoid tissue,[66] CD4 T cells in the mesenteric lymph nodes,[67] intraepithelial lymphocytes in the duodenum,[68] and B cell blasts in the lamina propria,[69] without being deleterious to CD4 T cells. This changes with HIV infection. We think that CD38 expression becomes pathogenic because it is overexpressed, its overexpression is chronical, and, importantly, it adds to the direct effects of HIV particles on CD4 T cells. Also delimiting our proposal, the naive subsets of CD4 AND CD8 T cells show lower activation in HIV infection (implying lower CD38 expression),[70] and their degree of activation is not predictive of HIV disease progression.[71] Moreover, they express CD38 constitutively.[72] Therefore, our proposal may be more relevant to memory CD4 T cells in HIV infection.

There is, to our knowledge, one study with results apparently opposite to our proposal. It finds a possible role of CD38 on *in vitro* protecting CD4 T cells from direct HIV infection. This conclusion was reached, based on differential infection of cell lines with different degrees of CD38 expression.[73] This work did not study important infectivity determinants like HIV coreceptors CCR5 and CXCR4, and it did not investigate primary CD4 T cells, precluding inferences about possible *in vivo* effects. In any case, this suggested role of CD38

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does not seem to be relevant to HIV disease progression, given the robustness, consistency, and abundance of studies demonstrating the positive correlation of CD38 expression with CD4 T cell loss *in vivo* (see Introduction). The role we propose for CD38 constitutes an indirect pathogenic mechanism. There is evidence suggesting that indirect mechanisms are necessary to fully explain CD4 T cell dysfunction and loss in HIV disease.[74,75]

3.3 Enhanced CD38 catalytic activity, possible effects on CD4 T cells

3.3.1. CD38-induced reduction of NAD content of CD4 T cells in HIV infection

—A possible consequence of excessive CD38 is an increased removal of its substrate, NAD, from the cytoplasm of T lymphocytes. This immediately raises the question of whether and how extracellular CD38 activity could regulate intracellular NAD concentrations.

Genetic CD38 ablation in CD4 T memory cells increases *in vivo* survival capacity, via an increased NAD cytoplasmic concentration.[53] Notably, this effect can also be attained by the use of an anti-CD38 antibody that blocks CD38's NADase activity. Antibodies can only block extracellularly-oriented catalytic domain of CD38; therefore, cytoplasmic NAD reduction in this model depends at least in part on extracellular CD38 activity. Importantly, the reduction of surface CD38 on these CD4 T cells resulted in higher cytoplasmic NAD concentrations, with a shift to enhanced oxidative phosphorylation.[53] This finding is of great relevance to our model, since extracellular CD38 is the form detected in standard phenotyping[72] and it is the one that strongly correlates with HIV disease progression. Thus, increased extracellular CD38 activity could translate in reduced cytoplasmic NAD. This has important consequences in cellular metabolism and senescence.

There are particular mechanisms that may mediate reduction of cytoplasmic NAD concentration by extracellular CD38 catalytic activity. NAD can be transported from the cytoplasm to the extracellular space by Cx43 connexin in an equilibrative flow,[76] and T cells express a surface functional Cx43.[77–80] Blood NAD concentrations range from 24 to 41.4 μM,[81–83] while the intracellular concentration ranges from 200 to 500 μM, and it may increase ten-fold.[64] This could generate a NAD gradient that, in the presence of membrane Cx43, could sustain a flow of NAD from the cytoplasm to the extracellular milieu. CD38 overexpression in HIV infection could translate in reduced NAD concentration in plasma and/or in T cell cytoplasm, increasing NAD flow from cytoplasm, and surpassing the cell's capacity to maintain its NAD levels. In consonance with a possible NAD scavenging pressure on intracellular NAD pools, we have found overexpression of nicotinamide phosphoribosyl transferase mRNA (NAMPT), the rate limiting enzyme of the salvage pathway of NAD synthesis) in central memory CD4 T cells from persons living with HIV,[84] which might be compensating NAD removal due to CD38 overexpression. In this way, increased presence of CD38 with an extracellular active site might decrease cytoplasmic NAD concentration. In agreement with this possible mechanism, cytoplasmic NAD maintenance depends more on the intracellular NAD synthesis via a salvage pathway, than in the intake of NAD,[64] while NAD can be transported outside the cell.

There is currently scant information of NAD contents of CD4 T cells from HIV^{+} individuals. An early study compared nucleotide intracellular concentrations in whole T cells (which include both CD4 and CD8 cells), from 8 healthy controls, 4 symptomatic patients, and 4

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asymptomatic patients.[85] Interestingly, they find a failure to increase intracellular NAD in response to *in vitro* activation in T cells from symptomatic patients, while basal concentrations did not differ between groups. However, no distinction can be made between CD8 and CD4 T cells, and inference is limited by the small sample. Therefore, further investigation of NAD levels in plasma and cytoplasm of CD4 T cells from HIV$^+$ patients and controls will be relevant for testing our hypotheses.

3. 3. 2. Low activity of Sirtuins and PARPs in CD4 T cells in HIV infection—If NAD overexpression is reducing intracellular NAD of CD4 T cells, as it does in other cells,[52] it may have important consequences. NAD has important functions in addition to its well-known biochemical role as an electron transporter in glycolysis, tricarboxylic acid cycle, and electron transfer chain. These functions include regulation of gene expression by sirtuins (Sirts), NAD-consuming deacetylases, and transfer of ADP-ribose groups from NAD to acceptor proteins by Poly (ADP-ribose) polymerases (PARPs), a process necessary for the regulation of cell death (reviewed elsewhere[86]). Sirtuins and PARP activities have important roles in metabolism, inflammation, DNA repair, and cell survival.[64] It is worth noting that nuclear Sirts 1 and 6 inhibit glycolysis and promote mitochondrial function, by promoting expression of PGC-1α,[87] and inhibiting HIF-1α and NFκB,[88] respectively (Figure 2). Likewise, mitochondrial Sirts 3 and 4 inhibit glycolysis and promote mitochondrial function, with Sirt 3 inhibiting generation of reactive oxygen species (Figure 2).[89] Cytoplasmic NAD is transported to mitochondria, and constitutes the main NAD source for this organelle,[90] which lends support to the possibility that a decrease in cytoplasmic NAD may reduce mitochondrial NAD content, along with decreases in Sirt 3 and Sirt 4 activity. Sirt 1, a nuclear deacetylase, promotes mitochondrial biogenesis and ROS protection.[87,91] Overall, NAD depletion affects mitochondrial integrity and function, thus reducing cell viability.

3. 3. 3.- HIV infection and Warburg effect in CD4 T cells—Quiescent T cells have a metabolism dominated by mitochondrial oxidative phosphorylation, beta oxidation of fatty acids providing the main carbon source for mitochondria. Importantly, pyruvate produced by glycolysis can be transported to the mitochondria, where it is converted to acetyl-coenzyme A, and contributes to the tricarboxylic acid cycle[92] (Figure 2). In contrast, activated T cells undergo Warburg effect.[93] In the case of T cells, this effect is described as an increase in aerobic glycolysis and a relatively reduced mitochondrial respiration after activation, which is reflected by a reduced oxygen consumption rate relative to extracellular acidification rate (OCR/ECAR ratio).[94,95] This shift may affect mitochondria fusion and cristae integrity[94] and yields an increased glucose intake and secretion of lactate.

Chronically activated CD4 T cells in HIV disease show increased aerobic glycolysis, as demonstrated by an increased frequency of cells expressing the glucose transporter Glut1, an increased glucose intake, an increased synthesis of glucose-6-phosphate from glucose, and an increased lactate secretion.[96] Importantly, the percentage of activated CD4 T cells (co-expressing CD38 and HLADR) was greater among Glut1$^+$ cells, which showed an increased density of surface CD38.[96] This could be reflecting an activation-associated Warburg effect, or a similar metabolic change, as has been noted in the case of monocytes from HIV$^+$

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patients.[97] Since this study did not measure mitochondrial respiration, which would fully demonstrate Warburg effect, a full metabolic profiling of CD4 T cells from persons with HIV and healthy donors is necessary (Figure 3) (Table 1).

3. 3. 4. Low CD4 T cell viability due to activation-induced Warburg effect—

Mitochondria integrity is related with the state of activation and is important for T cell viability. Loss of mitochondria integrity due to cytochrome oxidase ablation in CD4 T cells translates in a decrease in oxidative phosphorylation and a simultaneous increase in glycolysis.[98] Experimental increase in mitochondrial fission suffices to enforce an activated effector functionality in T cells, with a metabolic shift to aerobic glycolysis,[94] whereas mitochondrial biogenesis and functionality (promoted by the activity of NAD-dependent Sirt 3[99]) is associated with long term survival of T cells.[53,100,101] We think that an increase in CD38 catalytic activity may yield a decrease in cytoplasmic and mitochondrial NAD, leading to a metabolic shift to Warburg effect, which in turn would reduce mitochondrial function and biogenesis. We propose that this mechanism may be present in CD4 T cells in HIV infection (Figure 3). This hypothesis can be tested by measuring mitochondrial biogenesis in CD4 T cells from HIV^+ patients and uninfected controls, and by the determination of mitochondrial respiration (Table 1). Regarding this objective, a crucial test will be to measure the oxygen consumption rate in proportion to the extracellular acidification rate (OCR/ECAR ratio[53]). In this regard, it will be useful to study the same differentiation subpopulations to rule out differences due to diverging degrees of differentiation between samples. Variables affected by differentiation include cytokine production, viability, proliferative capacity, differentiation capacity, and metabolic profiles.[72,102,103]

3. 3. 5. CD38-products ADPR and cADPR increase cytoplasmic Ca^{2+} in CD4 T cells—

Independently of the final NAD balance outside and inside the cell, an increased turnover of NAD by CD38 would yield an increased production of cyclic adenosine diphosphate-ribose (cADPR) and adenosine diphosphate-ribose (ADPR), its two main products at neutral pH. Cyclic ADPR is an activator of the ryanodine receptor (RyR) calcium channel, able to trigger Ca^{2+} flow from the endoplasmic reticulum to the cytosol.[104] ADPR is the only known activator of the transient receptor potential melastatin 2 calcium channel (TRPM2).[105] TRPM2 channels are situated in the cytoplasmic membrane and transport Ca^{2+} from the cell exterior to the cytoplasm. ADPR and cADPR act intracellularly, which also raises the question of whether and how CD38, with its catalytic site facing outside the cell, can increase cADPR and ADPR concentrations in the cytoplasm. Such autocrine and paracrine activities of extracellular CD38 would require that cADPR and ADPR are transferred to the cytoplasm to act on ryanodine receptors (RyR) and TRPM2, correspondingly, thus triggering release of Ca^{2+} from endoplasmic reticulum and import of extracellular Ca^{2+}.

An alternative to autocrine and paracrine cADPR and ADPR would be an intracellular origin of cytoplasmic cADPR, as has been shown in HL-60 cells, U937 cells, and human monocytes. In these cells, CD38 can exist as a type III protein with its catalytic domain towards the cytoplasm.[106,107] This type III CD38 produces intracellular cADPR in

HEK293 cells.[106,107] Nevertheless, there is also evidence of mechanisms by which extracellular cADPR and ADPR could reach their target Ca^{2+} channels in the cytoplasm. Of particular relevance to our proposal, in Jurkat cells (a CD4 T cell line), extracellularly oriented CD38 can be internalized and transport cADPR to the cytoplasm.[108] In 3TC fibroblasts, endocytic vesicles containing CD38 can also transport NAD, transform it to cADPR, and liberate it to the cytosol.[109] Extracellular cADPR can also be transported to the cytoplasm by the concentrative nucleoside transporter CNT2 in 3TC fibroblasts,[110] and in HL-60 cells.[111] Connexin-43 hemichannels can also internalize cADPR.[112] As stated before, this protein is expressed by CD4 T cells,[79,80] further suggesting the likeliness of our proposal.

Regarding ADPR, its transport by the cell membrane from the outer milieu to the cytosol is possible, although less evidence is available. ADPR uptake has been found in human erythrocytes.[113] This uptake requires NAD glycohydrolase activity plus the presence of NAD, and was not observable by the mere addition of ADPR to the culture.[113] Red cell NAD glycohydrolase is likely to be CD38, since erythrocytes do not express CD157, the only other known cell surface NADase.[114] ADPR transport may involve a dual activity of CD38, synthesizing ADPR and transferring it to the cell. ADPR uptake has been verified, with the additional finding that some ADPR was incorporated to the cell membrane.[115] Interestingly, in the latter study transport was inhibited by ADPR but not by NAD, suggesting that only recently synthesized ADPR was transported by CD38. Addition of extracellular ADPR increases the intracellular Ca^{2+} concentration of human monocytes, also suggesting ADPR transport to the cytosol.[116] These mechanisms must be thoroughly studied in CD4 T cells.

3. 3. 6.- Reduced CD4 T cell viability due to increased cytoplasmic Ca^{2+}—

As a final aspect of our model of CD38-promoted CD4 T cell dysfunction and depletion, chronically increased cytoplasmic Ca^{2+} concentration elicited by cADPR or ADPR may be detrimental CD4 T cells, even though it has physiological functions (for an example see[117]). Calcium release from the endoplasmic reticulum can in some conditions activate the mitochondrial pathway of apoptosis, mediated by mitochondrial permeability transition and liberation of Cytochrome C, which promotes apoptosis[118,119] (Figure 4). It will be necessary to compare Ca^{2+} concentration in CD4 T cells from HIV^{+} patients and healthy controls, as well as to compare calcium flux upon TCR engagement (Table 1). We would expect these variables to be increased in cells bearing more CD38 molecules. Likewise, measuring the levels of cADPR and ADPR in plasma and cell culture media from patients and controls would further evaluate this possible mechanism (Table 1). Studying CD4 T cells from patients and controls from a same differentiation subpopulation (for instance, central memory CD4 T cells) is necessary to rule out differences due to diverging degrees of differentiation between samples. Variables affected by differentiation include cytokine production, viability, proliferative capacity, differentiation capacity, and metabolic profiles.[72,102,103]

If our hypotheses are verified in CD4 T cells in the setting of HIV infection, CD38 antagonists could be used as a therapeutic tool, adjunct to antiretroviral therapy. Such antagonists have already been proposed for other clinical applications,[120] which in

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principle makes such application feasible. A particularly interesting possible target tissue of CD38 inhibition is gut-associated lymphoid tissue, in which CD4 T cell recovery under antiretroviral therapy is particularly limited, coincident with a high level of immune activation (with its associated increase in CD38 expression[58,59,62]).

4. Conclusions and outlook

We provide background supporting the pertinence of asking whether CD38 overexpression facilitates CD4 T cell depletion in the setting of HIV infection. We describe existing evidence of the enzymatic function of CD38 and its effects in different cells, supporting proposed mechanisms that could mediate an indirect pathogenic role of chronic over-expression of CD38 in HIV infection. Chronic increased activity of CD38 in the milieu of memory CD4 T cells may increase NAD turnover, causing cytoplasmic NAD depletion. This in turn can promote Warburg effect, affecting mitochondria function and integrity. Additionally, CD38 catalytic products may increase cytoplasmic Ca^{2+} concentrations, which may further increase mitochondrial stress, ultimately leading to lower survival of CD4 T cells. This series of hypotheses is amenable to experimental testing. If verified, they may provide a new therapeutic strategy as adjunct to antiretroviral therapy.

Acknowledgements

Funding: Mexican National Council for Science and Technology (CONACYT) FC-2015-214 (E. Espinosa), The National Institutes of Health grants AI091526 (W. Jiang) and AI128864 (W. Jiang). We gratefully thank María-José Flores for language editing.

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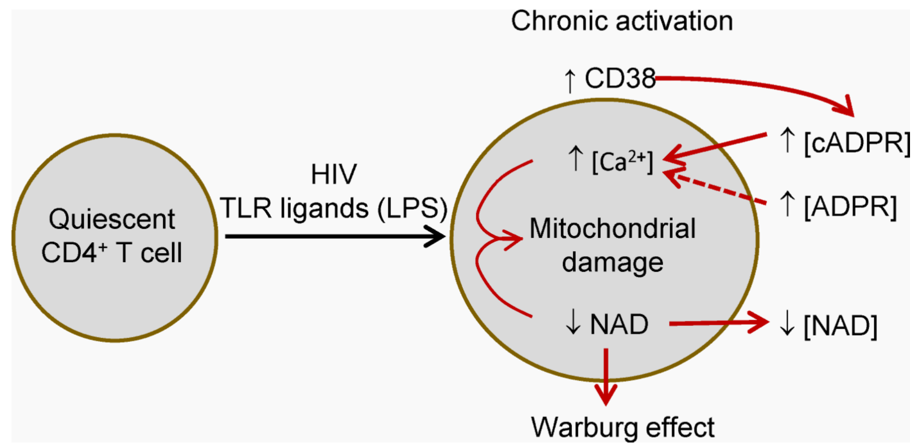

Figure 1.- General hypothesis outline.

HIV infection is accompanied by chronic activation of CD4 T cells and CD8 T cells (among others). This increases CD38 catalytic activity in the milieu of CD4 T cells. CD38 activity removes cytoplasmic NAD from CD4 T cells and yields the calcium-mobilizing compounds cyclic adenosine diphosphate ribose (cADPR) and adenosine diphosphate ribose (ADPR). cADPR and ADPR increase cytoplasmic Ca^{2+}. Together, these effects reduce mitochondrial function and integrity, leading to a decreased cell viability in the long term. Dotted line indicates that less evidence is available.

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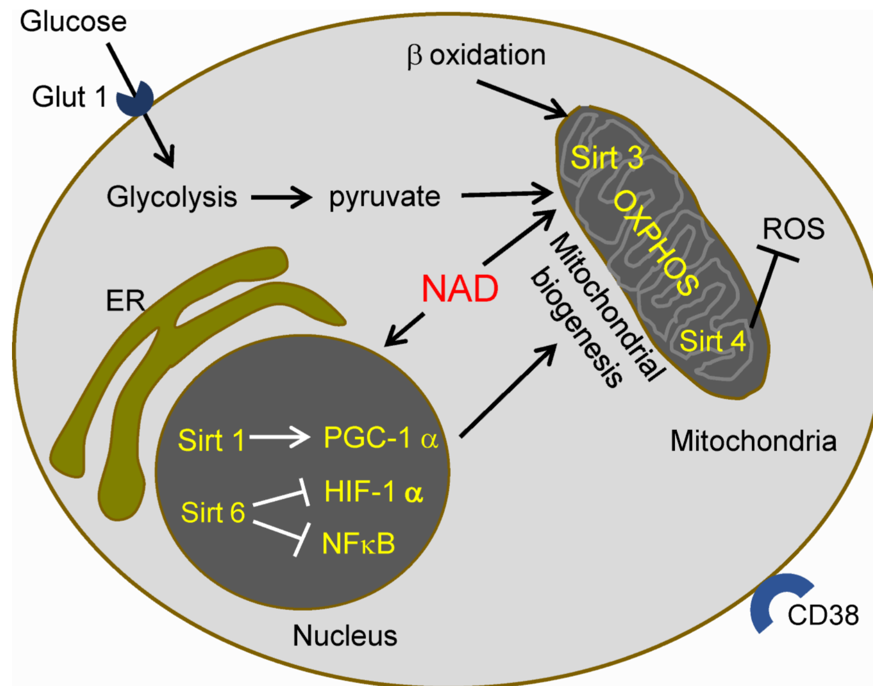

Figure 2.- Metabolic profile of quiescent CD4 T cells.

Quiescent CD4 T cells have a metabolism dominated by oxidative phosphorylation (OXPHOS) in mitochondria. OXPHOS is fueled by pyruvate from glycolysis and by β oxidation of lipids. This metabolic program is enforced by the activity of NAD-dependent Sirtuins (Sirt). Nuclear Sirtuin 1 induces PGC-1, which increases mitochondrial biogenesis. Sirtuin 6 suppresses HIF-1 α and NFκB, decreasing glycolytic metabolism and effector functions. Mitochondrial Sirt 3 promotes OXPHOS, and Sirt 4 lowers the production of reactive oxygen species (ROS). ER, endoplasmic reticulum. NAD, nicotinamide-adenine dinucleotide. Glut1, glucose transporter 1.

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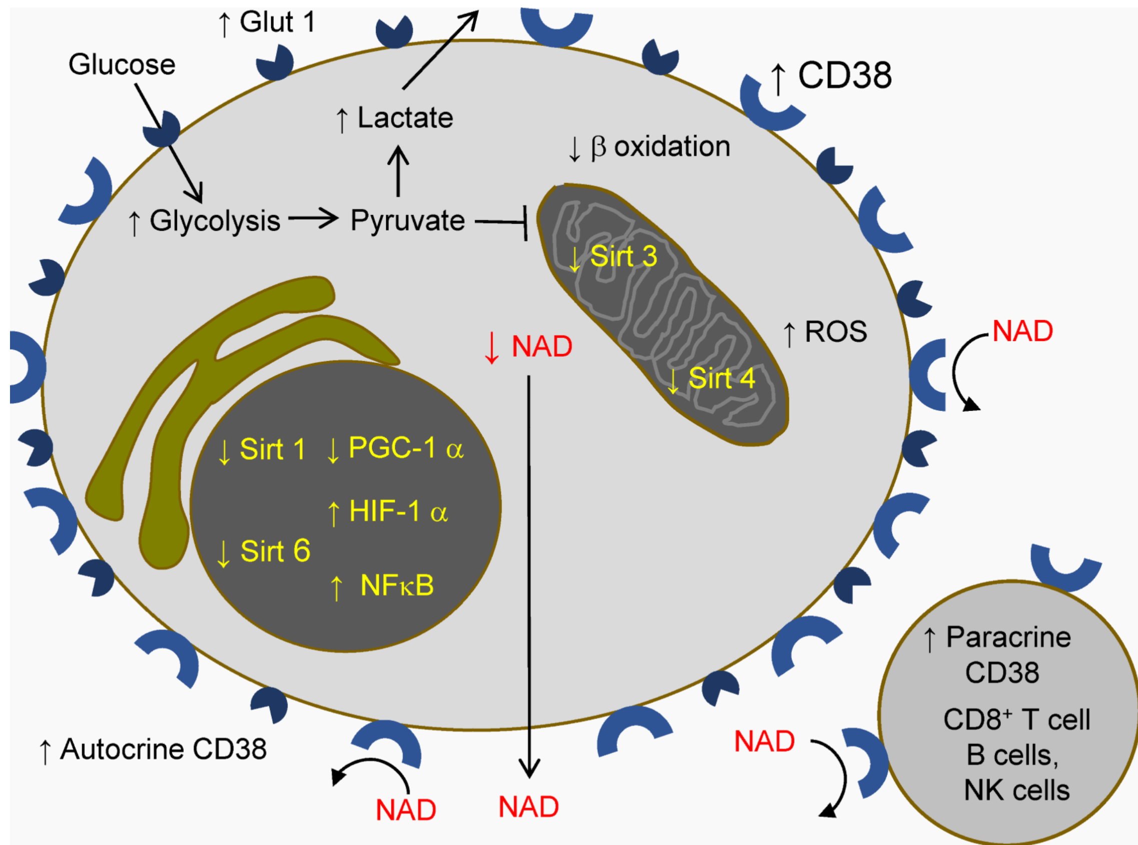

Figure 3. Activation and Warburg effect in CD4 T cells in HIV disease.

T cell activation increases CD38 expression, which increases catalytic activity on T cells, and in their homing sites. NAD removal by CD38 inactivates sirtuins, which decreases oxidative phosphorylation via the inhibition of PGC-1 and the activation of HIF-1 alfa. This blocks the entrance to mitochondria of pyruvate and products of lipid beta oxidation. NAD depletion additionally decreases the use of lipid beta oxidation products by mitochondria, via inactivation of mitochondrial Sirt3. Inactivation of Sirt 4 increases mitochondrial production of reactive oxygen species (ROS). Activation also increases CD4 T cell expression of the glucose transporter Glut 1, the entrance of glucose, and an increase in glycolysis. Pyruvate produced by glycolysis is not transported to mitochondria, but reduced to lactate, which is transported outside the cell. Warburg effect, entailing glycolysis-dominated metabolism, is thus enforced.

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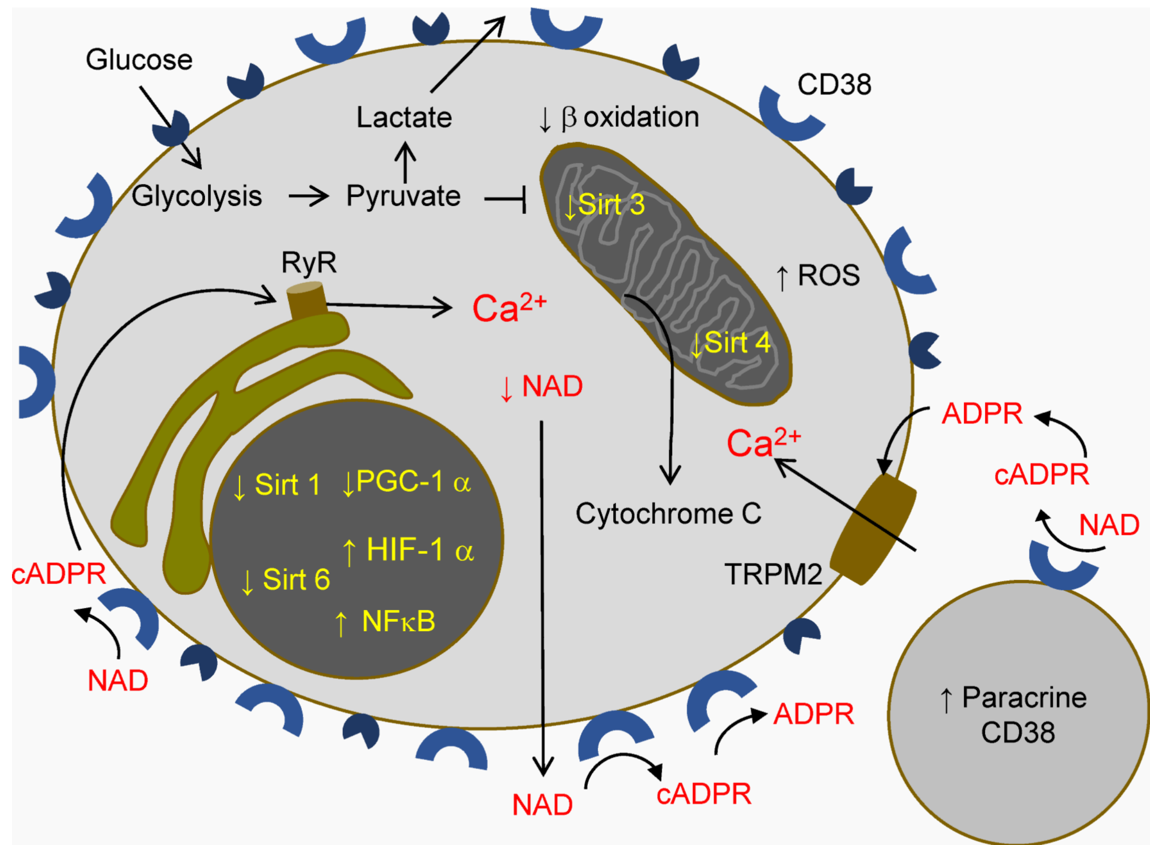

Figure 4.- Cytoplasmic Ca^{2+} increase in activated CD4 T cells in HIV disease.

Concomitant to Warburg effect, NAD turnover yields cADPR and ADPR, which can be transported to the cytoplasm and activate the calcium channels ryanodine receptor (RyR) on the endoplasmic reticulum, and Transient Receptor Potential Melastatin 2 channels (TRPM2) on the cell membrane, respectively. The ensuing increase in cytoplasmic Ca^{2+} further stresses mitochondria, which can cause the release of cytochrome C, a programmed cell death signal.

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Table 1.

Hypotheses conforming the proposed model of CD38-mediated indirect pathogenesis in HIV infection, and possible experimental approaches to test each.

Hypothesis	Possible experiments
2. 1 Catalytic activity where CD38 is present	Comparison of catalytic activity between $CD38^+$ and $CD38^-$ cells. Comparison of catalytic activity of CD4 T cells from HIV+ patients and healthy controls. Analysis of correlation of catalytic activity with CD38 expression. Possible methods: Degradation of NAD or an analog in culture media by colorimetry or HPLC-MS.
2.2 NAD depletion by CD38 activity	NAD measurement in lysates from CD4 T cells from patients and controls (colorimetry or HPLC-MS), and determination of its correlation with CD38 expression.
2. 3 cADPR- and ADPR production by CD38	ADPR and cADPR concentration determination in culture media and lysates of CD4 T cells from patients and healthy controls. Correlation with CD38 expression. Possible method: HPLC-MS
2. 4 Metabolic switch induced by NAD depletion	Determination of oxygen consumption rate (OCR, a measure of mitochondrial respiration) and extracellular acidification rate (ECAR, a measure of glycolytic activity) in CD4 T cells from HIV^+ patients and controls. Purified cells with the same degree of differentiation should be used*. Possible method: OCR/ ECAR ratio by mitocondrial stress and glycolytic stress kinetic determinations with available instrumentation and kits.
2. 5 Increased cytoplasmic Ca^{2+}	Comparison of basal Ca^{2+} levels in CD4 T cells from HIV^+ patients and healthy controls*. Analysis of correlation with CD38 expression. Comparison of Ca^{2+} flux after TCR-mediated stimulation in CD4 T cells from HIV^+ patients and healthy controls with the same degree of differentiation. *Analysis of correlation with CD38 expression. Effect of antagonists of cADPR (8-Br-cADPR) and of ADPR in cytoplasmic Ca^{2+} concentrations and functionality (see below) of CD4 T cells from HIV^+ patients and healthy controls*. Possible method: flow cytometry using a Ca^{2+} molecular probe.
2. 6. Mitochondrial damage	Mitochondrial morphology determination (e.g. mitocondrial fusion or fission and cristae integrity by confocal and electronic microspcopy)*. Mitochonrial biogenesis determination. Possible methods: microscopy and flow Cytometry with mitocondria-specific molecular probes*.
2.7 Altered survival, proliferation, cytokine production, and differentiation	Effect of pharmacological inhibition of CD38's catalytic function in proliferation, survival, differentiation, and cytokine production after stimulation of CD4 T cells from patients and controls *. Determination of functional properties of CD4 T cell lines (for instance, Jurkat cells) expressing functional or non-catalytic variants of CD38. Possible method: CRISPR-Cas9 deletion of CD38 gen, followed by transfection with plasmids codifying for CD38 variants with or without catalytic function. Functional tests may be carried out by flow Cytometry using fluorochrome-conjugated monoclonal antibodies, cell division molecular probes, and viabiity molecular probes.

*The use of CD4 T cells from patients and controls with a same degree of differentiation is proposed to rule out confounding effects of differentiation. Variables affected by differentiation include cytokine production, viability, proliferative capacity, differentiation capacity, and metabolic profiles.