La infección VIH conduce a una depleción severa de células T CD4+, alteraciones del fenotipo de las subpoblaciones linfocitarias, disminución de la función tímica que a su vez produce un deterioro progresivo de la función inmunitaria. La introducción de la terapia antirretroviral de gran actividad (TARGA) ha disminuido la progresión clínica, suprime la carga viral (CV) e incrementa las células T CD4+ en pacientes infectados por el VIH. Sin embargo, los niños en TARGA suelen tener CV más elevada y peor respuesta virológica que en adultos. No obstante, en los niños infectados por el VIH con bajos valores de células T CD4+, la TARGA produce un aumento consistente de células T CD4+ y de los llamados T-cell rearrangement excision circles (TRECs). Estos resultados indican que la recuperación de la función tímica juega un papel clave en la reconstitución inmunitaria de los niños con VIH. Entre las citocinas identificadas como posibles reguladoras de la timopoyesis, la IL-7 puede jugar un papel esencial. IL-7 participa en la diferenciación de los timocitos a células T virgen maduras en el timo que posteriormente salen a sangre periférica. Varios trabajos han demostrado la correlación inversa entre niveles plasmáticos de IL-7 y las células T CD4+. Por consiguiente, el aumento de IL-7 se ha propuesto como una respuesta homeostática a la depleción de células T CD4+. También se ha comprobado una inhibición de la timopoyesis por cepas virales X4 independientemente de la edad debido a la capacidad de las cepas X4 de infectar a los timocitos. Sin embargo se ha demostrado que la TARGA disminuye el número de aislados virales con fenotipo X4 en los niños con VIH. En conclusión, la TARGA ha mostrado ser eficaz disminuyendo la CV y aumentando el número de células T CD4+, proceso que depende de la función tímica y estaría regulado por IL-7.

PALABRAS CLAVE: VIH-1 / TARGA / Reconstitución inmunitaria / Timo / IL-7 / TRECs.

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Immune reconstitution in HIV-infected children on antiretroviral therapy
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RECONSTITUCIÓN INMUNITARIA DE NIÑOS INFECTADOS POR EL VIH BAJO TRATAMIENTO ANTIRRETROVIRAL DE GRAN ACTIVIDAD

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INTRODUCTION

Ever since AIDS pandemic started spreading worldwide, the numbers of vertically HIV-1-infected children have increased steadily. It has long been known that the natural course of HIV infection causes a progressive immune depletion characterized by an early decrease in the cellular response. Interestingly, HIV-infected children present some distinct features when compared to adults^1-4_. Thus, HIV infection in children presents a bimodal distribution: i) a short incubation period followed by a rapidly progressive course of the infection with severe immunodeficiency, featuring clinical symptoms of AIDS before the first 18 months of life; and ii) slow and chronic development of the disease similar to the pattern described for HIV+ adults^3-4_. Infected infants also experience high viral load levels for longer time periods^10_.

The reasons for these distinct features are still under investigation, but several explanations are gaining strength. On the one hand, HIV infection in the foetus or neonate occurs at a time when the immune system is under development and it is not fully competent. Potential target cells of the immune system are routinely stimulated and activated on exposure to a host of new antigens (whether natural or by immunization) representing an ideal setting for enhancement of viral replication. On the other hand, CD4^+ T-lymphocyte numbers are higher in infants than in school-age children and adults, potentially providing a larger target population for viral replication^16_. Infants and children present a major challenge for healthcare providers since they also possess specific metabolic functions that are absent in adults, which makes it difficult to define the optimal dosage and leads to variable responses to therapy. The poor palatability of many antiretroviral preparations and patients’ variable adherence to prescribed regimens are other important factors that add up to this challenge.

To date, most therapeutic efforts have been directed towards the development of novel antiviral agents that inhibit key viral enzymes and thus block the infectious cycle, along with the pursuit of effective immunization strategies. The implementation of antiretroviral therapies such as highly active antiretroviral therapy (HAART) has achieved a considerable reduction of the viral load and/or a suppression of the viral replication below detectable levels. This has lead to a dramatic descent in AIDS-related mortality rates and incidence of opportunistic infections in western societies^16_. Furthermore, the effectiveness of HAART in children to reduce HIV-1-related deaths is at least similar to, or even greater than, that observed in adults^10_.

However, HIV infection is still incurable and the AIDS pandemic progresses world-wide, particularly in developing countries. The underlying causes of this failure are still under debate. On the one hand, a pool of latently infected, memory CD4^+ T lymphocytes and other cell types may constitute a reservoir for the virus, which would thus remain inaccessible to administered antiretroviral agents^8,13_. On the other hand, the appearance of viral mutants resistant to the limited number of currently available drugs also diminishes therapeutic efficacy. As a result, no current therapy is completely successful and the immune system therefore becomes progressively impaired. A failure of thymic cell production and peripheral homeostasis may also be key elements in interfering in the interplay between different parts of the lymphoid compartment once this virus has induced severe damage.

Thus, immune reconstitution sensu stricto seems unattainable at present. Therefore, seeking acceptable degrees of immune reconstitution that lead to a chronic infectious status rather than a rapidly fatal outcome is still one of the main goals. In this regard, current therapeutic regimens with HAART accomplish a moderate degree of recuperation in terms of an increase in T cell counts and functionality, and a decrease in viral replication and other parameters^22_. With respect to the effect of HAART on humoral immunity, there are contradictory results. Several studies report decreases in neutralizing antibodies titres after ART^16,17_, but others have described either no effect^10_ or increased neutralization titres after therapy^18_. HAART treatments fail to provide viral eradication and major reconstitution of the impaired HIV-specific T-cell responses. Thus, patients do not generally become long-term non-progressors and thus cannot hope for long-term discontinuation of drug therapy. Recent studies in HIV-infected children on HAART show immunological recovery despite virological failure, regardless of the presence of circulating HIV-1 resistant mutants^17_.

Nevertheless, the striking similarities observed in HIV infection in children and adults have justified the prescription of similar therapeutic approaches. Despite the most recent treatment strategies modifying the clinical appearance of this infection in children, major challenges remain regarding this vulnerable sector of the population that faces long-duration aggressive treatments and drug switches that may lead to severe side-effects. Broadly, immune reconstitution in HIV-1-infected children on antiretroviral therapy may result from the de novo generation of T cells from the thymus, peripheral T-cell distribution and/or expansion, and decreased T-cell destruction. This review focuses on the mechanisms underlying this immune reconstitution that we are currently able to attain, bringing together some of the latest insights into what takes place...
regarding the specific cellular response to HIV, the role of the thymus and the homeostatic mechanisms with IL-7(20).

**SPECIFIC CELLULAR RESPONSE TO HIV**

The main hallmark of HIV-1 infection is the progressive elimination of primary CD4+ T cells. In HIV+ individuals, CD4+ T cell half life is substantially shortened (from 82 days in uninfected controls to 23 days in infected patients)(30). CD4+ T cell lymphopenia is due to a shortened T cell survival time, along with a failure to increase circulating T cells and a failure to generate functional T cells. However, the extent and nature of this cellular depletion are still under debate. The recovery of T-cell levels may arise out of a number of different mechanisms, including redistribution or peripheral expansion of pre-existing cells, prevention of apoptosis, less activation, etc. Therefore, the locations and mechanisms involved in the production of CD4+ T cells are key issues for understanding and promoting the appropriate reconstitution of the immune system(20, 21).

Since the cytopathic effect of HIV on infected CD4+ cells is unlikely to provide a satisfactory explanation on its own for the cause of this disease, several other types of immune cells are thought to play an essential role. Among these, CD8+ T lymphocytes play a dual function in the specific cellular response to HIV. They are responsible for the removal of virally infected cells and are also crucial in triggering and maintaining specific immune responses to HIV throughout the chronic infection phase(22, 23). Ever since CD4+ cells become infected by HIV, the progressive decrease in their number that ensues is the key for the development of the disease(20). Interestingly, in spite of the normal CD4+ cell counts that may be recorded at earlier stages of HIV infection, functions such as their ability to proliferate also become impaired(25-28). Surprisingly, HIV-infected patients on ART may present appropriate CD4+ responses to other viruses such as cytomegalovirus(29).

Therefore, HIV-1-specific immune response thus does not seem to be recovered after ART (Fig. 1). In fact, patients on ART feature HIV-specific CD4+ T-cells with effector functions, but their precursors are selectively destroyed right from the beginning of the infection(20, 22). A possible explanation of this selective cell removal could be the massive viral replication that takes place inside them. The ensuing release of high levels of viral antigens would quickly consume the HIV-specific, memory CD4+ T-cell pool, in a helpless attempt of the organism to control the infection(30). The absence of these cooperating cells or precursor «helper cells» would then lead to a progressive decrease in subpopulations of HIV-specific, CD4+ T cells. This, along with viral-induced cell depletion, would account for the dramatic decrease of CD4+ T cells seen in these patients. The absence of these HIV-specific CD4+ T cells also leads to insufficient production of certain soluble factors essential for the maturation and survival of effector CD8+ T cells.

All these data advocate for the administration of HAART right from the earlier phases of the primary infection, in order to preserve the specific «helper» CD4+ T-cell response(32). It has been shown that CD4+ T-cell pre-treatment lows determine the recovery of immune responses(12, 34). Besides, both phenotypic and functional immune restoration remain incomplete in spite of the normalization of circulating CD4+ T-cell counts if initiation of ART is delayed(12, 32, 34-38). These data confirm that a CD4+ repertoire that is largely impaired by the infection, as occurs in advanced stages of the disease, has a small probability of to be regenerated after ART. It is known that the reconstitution of T-cell numbers in the first two years after chemotherapy-induced depletion follows a particular pattern: whereas the reconstitution of CD8+ T-cell numbers is rapid and occurs through peripheral expansion, the recovery of CD4+ T-cells is limited and delayed, being constrained by the age-dependent decline in thymopoiesis. In this regard, successful CD4+ T-cell reconstitution is determined to a large degree by the thymic output(39). In contrast, CD8+ T-cell reconstitution is not dependent on age nor on thymic output(40).

During chronic infection in adults, the number of virus-specific CD8+ T-cells often exceeds that of virus-replicating infected cells. This suggests a failure of their cytolytic properties, apparently linked to defects in perforin(41), defective signalling due to CD3x and CD28 down-modulation(42), a skewed maturation pattern(43) and/or ineffective trafficking to sites of infection. Most antiviral CD8+ T-cells found in chronic infection do not express perforin, a molecule required for cytolysis. CD8+ T-cells can rapidly lose their effector cytotoxic function, independently of CD4+ T-cell depletion(44). Controlling viral replication with drugs, particularly during the first weeks or months after infection, reduces the viral burden and could thus block the development of T-cell anergy(45).

Infected patients who are following effective ART regimens normally experience an increase in CD4+ counts. The initial rise in circulating CD4+ cells is probably due to redistribution of cells previously trapped by the large amount of viral antigen in lymphoid tissues(44-46). This is followed by a gradual increase in naive CD4+ T-cells, possibly owing to a combination of peripheral expansion and continued thymic production of T-cells. In addition, ART decreases virus-induced immune activation(44, 45, 47), which in turn may lead to decreased death of activated T lymphocytes. Thus, a
rapid and remarkable reduction in the cell surface expression of T-cell activation markers occurs in parallel with control of virus replication(44, 46-49). Similarly, levels of plasma inflammatory cytokines TNF-alpha and IL-6 also fall. Both phenomena may help to reduce the abnormal rate of cell death observed during the natural course of HIV infection in untreated patients(50) and to restore normal numbers in the T-cell compartments(51). In our experience, comparable results are found in children. However, there are differences between adults and children with respect to the phenotype of the T-cell that appears during this process of reconstitution. In children, 75% of the CD4+ increase is attributable to the naïve CD45RA+ population(52), whereas in adults a CD45RO+ population predominates(44, 45), suggesting a thymic origin of the CD4+ recovery in HIV-infected children(53).

Interleukin-2 has been utilized as immunotherapy to improve the recovery of T-cells, as its production and receptor expression have been shown to be defective during the course of disease(54, 55). There are several successful clinical trials showing that IL-2 leads to dramatic increases in CD4+ T-cell numbers(56-58). In addition to increasing both naïve and memory T-cell numbers and function, IL-2-based immunotherapy in conjunction with HAART is associated with a greater reduction in viral load than that seen with antiretroviral therapy alone in patients with baseline CD4+ T-cell counts of >200 cells/µl(59). Recent studies using intermittent IL-2 therapy in conjunction with HAART show that in addition to at least a two-fold increase in CD4+ T-cell numbers and a reduction of viral reservoirs, small blips of vireemia occur, which may induce autovaccination and induction of HIV-1-specific T-cell responses(60-62). With respect to the effects of IL-2 in the thymic recovery of T-cells, there are contradictory results. Pido-Lopez et al. have shown that CD4+ T-cell reconstitution in patients receiving IL-2 with HAART is largely due to thymus-independent mechanisms, with the thymus making only a limited contribution(64). However, Carcelain et al. describe how the thymus plays an important role in the long-term recovery of naïve T-cells under IL-2 therapy(65).

ROLE OF THE THYMUS IN THE PEDIATRIC IMMUNE RESPONSE

The thymus is an essential organ for establishing a functional peripheral T-cell pool. Studies in mice indicate that naïve and memory CD8+ T-cell pools are independently regulated, with each pool of T-cells having its own niche. In humans, it is well established that HIV-1 infection adversely affects the thymus in both children and adults(40, 66). Yet the consequences of this thymic inhibition are worse in children, and this has been proposed as one cause of the rapid progression of the disease(46-49). Moreover, faster disease progression has been observed in children infected in the uterus than in children infected intrapartum, probably as a consequence of the early infection of the thymus(70). Furthermore, even exposure to the virus affects the normal development of the immune system. Smaller thymus glands, as determined by sonography, have been observed in uninfected-children born to HIV-positive mothers(71), and a persistent mitochondrial dysfunction has been also associated with the exposure of the virus in non-infected children(72).

Early ART in HIV-infected children has proven to induce an early immune reconstitution that correlates with the increase in thymus volume(72). ART has proven to be effective in recovering appropriate CD4+ T lymphocyte levels in HIV-infected patients with low CD4+ T lymphocyte counts(56, 59), possibly through the production of new T-cells from the thymus. This is particularly relevant for children, who have a smaller T-cell pool and thus rely upon their thymic output for the generation of immune-competent memory T-cells. In this regard, a repopulation originating in the thymus would allow the recovery of the complete repertoire of specificities, including HIV-specific ones. This contrasts with the effects of the redistribution or peripheral expansion that would only increase the number of cells of those clones that have survived the infection without recovering specific responses to opportunistic pathogens and HIV.

However, the peripheral expansion of post-thymic T-cells is neither efficient in reconstituting the peripheral T-cell pool nor in regenerating a complete T-cell repertoire(76, 77). Since a quantitative increase in the population of CD4+ T lymphocytes is not enough to reconstitute the immune system, a functional improvement is also needed. In this regard, adults and children must be considered separately when the role of the thymus is being assessed. In adults, the ability of the thymus to produce new T-cells throughout life progressively decreases with the natural involution of the gland(78). A standard method of assessing thymic T-cell production is to quantify TREC. Using this method, it has been shown that although an 80% reduction in normal thymic output occurs in all HIV+ patients regardless of their age, in adults the actual number needed to replenish the peripheral pool is much lower (Table I). Therefore, in adults the CD4+ T-cell recovery after treatment seems to have a peripheral origin. In fact, it has been shown that the level of peripheral T-cells in adults is maintained primarily by proliferation of the post-thymic peripheral T-cells(20, 79). In contrast, the child’s thymus preserves all its functionality and, in contrast to adults, the pool of peripheral T-cells in children is mainly maintained by thymopoiesis(76, 81), which...
In the context of HIV-infected children on antiretroviral therapy (ART), the thymus plays a crucial role in lymphocyte reconstitution. The thymus is a lymphoid organ responsible for the development of T lymphocytes, which are essential for the immune system's function. In children, thymopoiesis, the process of thymic production, correlates well with the recovery of CD4+ T-cell counts, allowing the production of new T-cells that would reconstitute the CD4+ T-cell pool lost after infection.

However, the effects of HIV on the thymus vary depending on the stage and severity of the infection. Children, especially those under 5 years of age, have a more immature immune system compared to adults, which makes them more susceptible to the effects of HIV. The thymus is crucial for the maturation of the immune system, rendering it more susceptible to the effects of HIV.

Table I presents the estimated thymic output of recent thymic emigrants in HIV-infected children and adults.

<table>
<thead>
<tr>
<th>Age quintile</th>
<th>0-1 year</th>
<th>2-10 year</th>
<th>11-25 year</th>
<th>26-49 year</th>
<th>≥ 50 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage thymic epithelial space</td>
<td>93%</td>
<td>88%</td>
<td>63%</td>
<td>45%</td>
<td>18%</td>
</tr>
<tr>
<td>Estimated daily thymocyte output in normal subjects*</td>
<td>≥ 10^9</td>
<td>8.8 x 10^8</td>
<td>6.3 x 10^8</td>
<td>4.5 x 10^8</td>
<td>1.8 x 10^7</td>
</tr>
<tr>
<td>Estimated daily thymocyte output in HIV-1 infected patients*</td>
<td>2 x 10^8</td>
<td>1.8 x 10^8</td>
<td>1.3 x 10^8</td>
<td>9.0 x 10^7</td>
<td>1.8 x 10^7</td>
</tr>
</tbody>
</table>

* TRECs levels in normal T-cells in peripheral blood CD4+ and CD8+ T-cells and HIV-1+ CD4+ and CD8+ T-cells. Reduction of normal output based on measured peripheral blood TRECs levels in HIV-1+ patients.

The decrease in circulating CD4+ T lymphocytes is more dramatic in HIV-infected children than in adults. Children undergo a combined effect of the natural descent of CD4+ T cells occurring with age together with CD4+ depletion owing to the HIV infection itself. This decrease affects thymic production, which is regulated by the thymus and the thymic hormones. The decrease in thymic function may seriously compromise the subsequent maturation of the immune system, rendering it more susceptible to the effects of HIV.

The thymus maintains a balance between the production of new T-cells and the destruction of thymocytes. This balance is crucial for the immune system's function. The thymus has been reported to be more susceptible to the effects of HIV than other lymphoid organs. The thymus has a higher turnover rate, allowing for a more rapid response to infection.

In conclusion, the thymus is a crucial organ for the reconstitution of the immune system in HIV-infected children. The thymus must be considered in the management of HIV-infected children on ART, as it plays a key role in maintaining the child's immune function.
microenvironment favours the replication of X4 variants by positively modulating the expression of CXCR4 in thymocytes. Zamarchi et al. describe how CXCR-4 was constitutively expressed by thymocytes (aprox. 10000 molecules/cell in about 30% of thymocytes), unlike CCR5, which presents a significantly lower expression (<4000 molecules/cell in less than 5% of thymocytes). This accounts for the greater fall of CD4+ lymphocyte levels in children after the appearance of these strains and highlights the key role played by the child thymus in the maintenance of appropriate CD4+ T-cell levels. The influence of the type of viral strain (X4 or R5) on cell counts happens to be less prominent in adults. This could be understood if we assume that the thymic T-cell production in adults is not a major determinant of T-cell numbers and that the reduced number of potentially infected thymocytes is due to thymic involution.

ROLE OF IL-7 ON HOMEOSTATIC MECHANISMS OF CD4+ T CELLS

As mentioned previously, three mechanisms allow CD4+ recovery: the redistribution of memory CD4+ T-cells from tissues, the regeneration of naive T-cells from the thymus, and a reduction in the inflammatory syndrome. This early reconstitution therefore does not solely reflect memory T-cell proliferation. Indeed, the early increase in CD4+ T-cells involves activated memory CD45RO+ T-cells that do not incorporate proliferation markers. Therefore, it is thought that sequestered T-cells in lymphoid tissues are released in blood when viral replication is controlled. However, whereas mature T-cells in the periphery seem to be able to adjust their proliferation and death rates to maintain numerical homeostasis during normal aging, it is not clear whether this is the case in HIV infection. There is still some debate regarding the thymic response to the depletion of the peripheral T-cell compartment. In fact, the recovery of CD4+ T-cells in children after antiretroviral therapy supports arguments for the existence of some homeostatic mechanism that detects low levels of CD4+ and activates the mechanisms needed to achieve cell repopulation. In our experience, the thymus plays a key role in this repopulation. Therefore these homeostatic mechanisms are likely to lead to an increase in the thymic production of new T-cells. One of the candidates to play a key role in this mechanism is interleukin-7 (IL-7).

IL-7 is a cytokine involved in the differentiation of thymocytes into T-cells that then leave the thymus and enter the peripheral blood. Furthermore, IL-7 has also proven essential in maintaining T-cell population in murine models and in inducing the reconstitution of the T-cell population in immunosuppressed mice. Interestingly, HIV-infected patients have abnormally high plasmatic levels of IL-7 associated with low levels of CD4+ T-cells. This suggests that low CD4+ counts should stimulate peripheral dendritic cells and lymphatic ganglia to produce IL-7, in an attempt to activate the mechanisms that allow cell repopulation (Figure 2). By contrast, other studies have suggested that plasma IL-7 levels may simply reflect the dynamics of binding
of secreted IL-7 to T-cells: the fewer circulating T-cells, the greater amount of free IL-7. However, these high levels of IL-7 are ineffective in adults since no increase in thymic production or recuperation of the CD4+ T-cell repertoire has been observed, possibly due to the limited ability of the adult thymus to produce new T-cells. In our experience, high levels of IL-7 in children with lower CD4+ T lymphocyte counts lead to a dramatic increase in the thymic production of new T-cells and a marked recovery of CD4+ counts. This increase in thymic function takes place whenever viral load levels are low enough. Later, on the recovery of CD4+ counts, IL-7 returns to basal values as a consequence of a feedback mechanism. This has also been reported in other types of lymphopenia, where high levels of IL-7 normalize whenever the lymphopenia itself resolves.

However, IL-7 increases the susceptibility of lymphocytes to become infected by HIV in vitro, inducing the expression of CXCR4 in dormant memory CD4+ T-cells. This means that high levels of IL-7 in vivo could have deleterious effects, leading to a selection of SI/X4 variants that could in turn foster the progression to AIDS. However, recent reports from infected macaques indicate that this is probably not the case and that IL-7 is not associated with increases in viral loads in either blood or lymph nodes. Other recent studies suggest that baseline IL-7 serum levels do not predict the immunologic outcome of children on HAART, and that immunologic failure occurs even in the presence of high IL-7 serum levels, thus suggesting that there are no benefits of therapy with exogenous IL-7. However, there are also data that suggest that therapeutic immune reconstitution in HIV-1-infected children is independent of their age and pre-ART immune status.

Much progress has been made in the therapy of pediatric HIV infection, which has been transformed from a usually fatal disease into a chronic disease. Efforts are under way to develop simpler, more effective therapeutic regimens that will suppress and ultimately eradicate the infection and that stimulate immune reconstitution. Clinicians caring for HIV-infected children are now considering the safety of discontinuing prophylactic therapies for children displaying sustained immunologic improvement on antiretroviral therapy. Despite these advances, physicians can expect HIV-infected children to continue to develop opportunistic infections and other related complications. Some children fail to respond to antiretroviral therapies, whereas others are unable to tolerate complex medication programs. Most children still require lifelong maintenance therapy in the absence of appropriate immune reconstitution.

We may conclude that the immune system in children has the potential ability to control HIV infection, triggering homeostatic mechanisms in charge of restoring lost cells and appropriate immune responses for fighting the virus both in blood and within cells. The data we have reviewed do not provide definitive answers but pinpoint promising pathways for improving the lives of these patients. A better understanding of the viral mechanisms and immune responses involved will certainly lead to novel approaches to the study of immunostimulatory therapies allowing an effective control of the infection by the immune system itself. This would eventually avoid the development of AIDS or even turn chronic HIV infection into a chronic asymptomatic disease with no consequences for the carrier’s health and an improvement in the child’s quality of life.

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REFERENCES


72. Barret B, Tardieu M, Rustin P, Lacroix C, Chabrol B, Desguerre


76. Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol 1996;156:4409-4416.


