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Population Dynamics of CD4$^+$ T Cells Lacking Thy-1 in Murine Retrovirus-Induced Immunodeficiency Syndrome (MAIDS)

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Increased numbers of CD4$^+$ Thy-1$^-$ cells have been described in the spleen (SP) of mice with retrovirus-induced immunodeficiency (MAIDS). Since this phenotypic abnormality might have considerable functional importance, the expansion of the CD4$^+$ Thy-1$^-$ subset in MAIDS was characterized further. CD4$^+$ Thy-1$^-$ and Thy-1$^+$ T-cells from infected mice expressed similar densities of CD3 and TCR α/β. In contrast, the Thy-1$^-$ subset was uniformly C'D44^{hi}, even early in the disease when part of Thy-1$^+$ cells were still CD44^{lo}, The emergence of CD4$^+$ Thy-1$^-$ cells occurred first in SP and lymph nodes and was observed later in thymus. The important fraction of CD4$^+$ cells lacking Thy-1 normally present in Peyer's patches was only weakly modified. Despite the major expansion of the CD4$^+$ Thy-1$^-$ phenotype. the proliferating fraction was not higher in this subset than in CD4$^+$ Thy-1$^-$ cells from infected mice. Persistence after hydroxyurea administration was identical in both subsets, indicating similar mean cell lifespans. Taken together, these results show that the major expansion of CD4$^+$ Thy-1$^-$ T-cells in MAIDS is not ascribable solely to increased proliferation within this subset. Phenotypic analysis suggests that CD4$^+$ Thy-1$^-$ cells result from the differentiation of Thy-1$^+$ cells induced by activation signals related to retroviral infection.

INTRODUCTION

Infection of susceptible strains of mice by viral isolates derived from the Latarjet-Duplan strain of radiation murine leukaemia viruses such as RadLV-Rs [1,2] and LP-BM5 MuLV [3-5] causes a lymphoproliferative disease with a profound impairment of immune responses [4-7]. T-cells participate in the lymphoproliferation and exhibit impaired responses to mitotiens *in vitro* [4-7]. However, the mechanisms responsible for the involvement of T-cells are unclear because most of the infected target cells belong to the B-cell lineage. It has been proposed that the defective retrovirus responsible for this murine acquired immunodeficiency syndrome (MAIDS) could act as an oncogene by stimulating a target B-cell population to proliferate [8-11]. In turn, this subset could affect additional cell populations through the production of soluble factors [12, 13] or the membrane expression of a viral product with superantigen-like properties [14, 15].

Abnormal expansion of a CD4$^+$ Thy-1$^-$ phenotype has been observed in SP and LN of mice infected with LP-BM5 MuLV [16]. This phenotypic abnormality is interesting as Thy-1 is associated with a protein tyrosin kinase and could be involved in signal transduction [17]. Thus, its absence on an important subset of T-cells might be related to the impairment of T-cell responses. However, al present it is not known whether these cells are the result of either a differentiation 01 activation disorder in T-cell lineage or if they represent the retrovirus-induced expansion of a minor CD4$^+$ Thy-1$^-$ T-cell population which exists in SP. LN and more prominently in Peyer's patches (PP) of non-infected mice [16, 18].

The kinetics of the CD4$^+$ Thy-1$^-$ phenotype expansion in thymus, SP, LN, PP and liver of infected mice was analysed. PP were considered because up to 40% of CD4$^+$ T-cells of this lymphoid organ are Thy-1$^-$ in normal animals [18]. Liver was studied because it has been reported that extrathymic differentiation of T-cells lacking Thy-1 takes place in this organ in the autoimmune mice with the MRL *lpr* mutation [19, 20] which develop a syndrome sharing some common features with MAIDS [21]. Also analysed was the expression of CD44 (PGP-1), a marker associated with the memory/activated stale induced by antigen-priming [22], within CD4$^+$ Thy-1$^-$ versus Thy-1$^+$ T-cell subsets early in the course of RadLV-Rs infection. Parameters defining population dynamics such as the proportion of cells in S and G2/M phases of the cycle and meat) cell lifespan were determined in each population. Although proliferative fraction was increased in both subsets of CD4^4 cells compared to controls, the results outlined below show that the preferential expansion of the Thy-1$^-$ phenotype is not due solely to increased proliferative activity of Thy-1 cells in any of the organs tested.

MATERIALS AND METHODS

Mice and cell suspensions

Male CS7B1/Ka (H-2b) mice were bred in the facility. Mice were injected twice i.p. at the age of 4 and 5 weeks with 0.25 ml RadLV Duplan MuLV stock solution. Aged-matched control mice were injected twice with 0.25 ml saline. After different time intervals (first injection, time 0). mice were sacrificed by CO_2 asphyxiation. SP. thymus. LN (inguinal, axillary, cervical, mediastinal and mesenteric) and PP were removed. Single-cell suspensions of LN, SP, PP and thymus were prepared separately with a fitting glass homogeneizer passed through a nylon cell strainer, washed three limes and counted on a Thoma haemacytometer. Hepatic lymphocytes were isolated according to Watanabe *et al.* [23].

Virus

Extract was prepared from the lymph nodes and spleen from three mice injected 2 months previously with RadLV-Rs extract 64. RadLV-Rs extract 64 was kindly provided by E. Legrand (INSERM 117 Bordeaux, France) and was described previously [1,2]. Lymphoid organs were ground in PBS and centrifuged 30 min at 1.5 $\times 10^4$ *g*. The supernatant was spun again for 30 min at 1.5 $\times 10^4$ *g*. This cell-free supernatant constituted the extract. It was injected immediately into mice or stored in liquid nitrogen. XC plaque assay [24] was used to measure virus litre. The virus preparation contained 1.0 $\times 10^3$ PPU of ecotropic virus/ml.

Antibodies

The MoAbs used were fluorescein isothiocyanate (FITC) conjugated anti-Thy-1.2 (30-H12). phycoerythrin (R-PE) labelled anti-CD4/L3T4 (GK1.5) (Becton Dickinson. Erembodegem, Belgium), biotinylated anti-mouse CD44 (IM7), biotinylated anti-mouse TCR *αβ* (H57 597) (Pharmingen, San Diego CA, USA), anti-CD3 MoAb (145-2C11) (provided by Dr J. A. Bluestone University ol Chicago, Chicago, IL, USA). Purified anti-Fc*γ*RII (CD32) (2.4G2) was purchased to Pharmingen (San Diego, CA. USA), 145-2C11 was conjugated to FITC or biotin following published protocols [25]. A purified rat IgG_{2b} myeloma (Gamma, Liège, Belgium) was used as isotypic irrelevant control for IM7, the anti-CD44 MoAb. Streptavidin-PE (Dako) and streptavidin-Red$^{613®}$ (Gibco BRL) were used as a second step reagent to reveal biotinylated antibodies.

Flow cytometry and cell sorting

Single-cell suspensions were prepared from lymphoid organs and stained with optimal amounts of MoAb on ice for 20 min in PBS with 2% 8SA and 0.1 % sodium azide. Cells were washed twice and counterstained with streptavidin-PE or slreptavidin-Red^{613}. After additional washes, cells were analysed on a FACStar Plus® (Becton Dickinson). Gating was performed according to forward and side scatter histograms. Data were collected and processed using the LYSIS II software (Becton Dickinson). Anti-Thy-1.2 (30-H12) induced significant doublet formation between Thy-1$^+$ T-cells and B-cells or macrophages from infected mice. Therefore, cell suspensions were preincubaled with anti-Fc*γ*RII (CD32) (clone 2.4G2) before the addition of anti-Thy-1.2 (30-H12). The complete absence of doublets after this treatment was confirmed by FSC analysis and fluorescence microscopy.

For sorting of CD4$^+$ Thy-1$^-$ and CD4$^+$ Thy-1$^+$ populations, cells were preincubaled with anti-Fc*γ*RII 2.4G2, stained as above with appropriate labelled antibodies and sorted on a FACStar Plus® (Becton Dickinson). Gates were set on FSC versus SSC dot plots(G1) (to eliminate dead cells, debris and non-lymphoid cells) and on FL1 (Thy-1-FITC) versus FL2 (CD4-PE) dot plots according to high level expression of CD4 and low (G2) versus high level (G3) expression of Thy-1. Logical sorting gales were used to combine both types of gates: G1 *and* G2 for CD4$^+$ Thy-1$^-$ cells; G1 *and* G3 for CD4$^+$ Thy-1$^+$ cells. Sorted cell populations were routinely 95-99% pure.

Cell cycle analysis

Cell cycle analysis was performed on isolated nuclei with the CycleTest™ kit (Becton Dickinson) according to Vindelov [26]. Briefly, cells were treated with a trypsin buffer and RNase. Isolated nuclei were incubated for 10 min with propidium iodide at room temperature, filtered and run in the Bow cytometer. For each sample 3×10^4-5×10^4 nuclei were analysed. The DNA histograms were analysed with the CellFIT™ software (Becton Dickinson) using the 'sum of broadened rectangles' model. This analysis provided statistical estimations for the three regions : G_0G_1, S and G_2M of the DNA histograms. The percentage of proliferation was calculated as the percentage of cells in S + G_2M phases of the cell cycle.

Hydroxyurea treatment

HU (Sigma) was dissolved in phosphate-buffered saline (PBS) and administered i.p. at 1 g/kg body weight in two separate injections with a 7-h interval according to the protocol of Hodgson *et al.* [27]. Control animals received two i.p. injections of PBS with a 7-h interval. Mice were sacrificed al various times after HU administration. LN were dissected and weighted. Cell suspensions were prepared from the pooled LN from three mice in each group.

RESULTS

Early expansion of the $CD4^+$ Thy-1^- phenotype in LN and SP but not in thymus, PP or liver

As reported previously with the LP-BM5 virus [15], RadLV-Rs Duplan infection was associated with the emergence of a $CD4^+$ T-cell population lacking Thy-1 in the SP and LN of infected animals (Fig. 1). This phenomenon was analysed sequentially in the LN, SP and thymus of infected mice (Fig. 2). The fraction of $CD4^+$ T-cells lacking Thy-1 was less than 10% in SP and LN of control animals. Their frequency was significantly lower in the thymus (less than 3% of total $CD4^+$ T-cells). No significant variation was observed after mock-i.p. injection (not shown). After i.p. inoculation of RadLV-Rs. the frequency of $CD4^+$ Thy-1^- cells increased rapidly in SP and LN and reached up to 60% of total $CD4^+$ T cells 8 weeks after infection. This frequency remained around 60% at least until week 15 after viral inoculation (not shown). In contrast with SP and LN, the appearance of $CD4^+$ Thy-1^- T-cells in the thymus of infected animals was much slower during the first weeks of infection. The process accelerated dramatically in the thymus later in the disease (week 7) and a frequency similar to that observed in SP and LN was reached eventually. To rule out the participation of a depletion of $CD4^+$ Thy-1^+ cells in the relative increase of Thy-1^- cells, the total cell numbers were calculated in LN $CD4^+$ Thy-1^+ and Thy-1^- cells. After RadLV-Rs inoculation, the absolute number of cells increased dramatically in each subset of $CD4^+$ T-cells and reached a plateau around week 8 post-inoculation. At this time point. the total number of cells was roughly equal in both subsets. Afterwards, the number of $CD4^+$ Thy-1^- T-cells remained constant while $CD4^+$ Thy-1^+ cells decreased slowly (unpublished observations). Compared to their respective values in controls, $CD4^+$ Thy-1^- T-cells displayed the more important expansion since their total number was increased 70-100-fold. In contrast the number of $CD4^+$ Thy-1^+ T-cells was increased only 8-12-fold (Table 1).

Table 1. *LN $CD4^+$ T-cell subsets in MAIDS. Total cell numbers*

	Week 4		Week 8	
	$CD4^+$ Thy-1^+	$CD4^+$ Thy-1^-	$CD4^+$ Thy-1^+	$CD4^+$ Thy-1^-
Controls	55.7	3.9	49.6	4.5
RadLV-Rs	153.8 ± 6.2	43.9 ± 6.8	341.4 ±51.8	350.5 ± 86.6

The data ($\times 10^6$) represent the LN $CD4^+$ T-cell numbers of two pooled control mice and the means ± SD of groups of live infected mice analysed individually.

Fig. 1. *Presence of $CD4^+$ Thy-1^- cells in a RadLV-RS infected mouse. LN cells from (A) a mock-injected control and (B) a RadLV-RS infected mouse (8 weeks post-infection) were stained with FITC labelled anti-Thy-1.2 and PE-labelled anti-CD4. In the control mouse $CD4^+$ Thy-1^- cells represent 2.22% of lymphocytes or 6.30% of $CD4^+$ cells; in the infected mouse they represent 19.48% of lymphocytes or 49.92% of $CD4^+$ cells. Similar dot plots were obtained with LN cells from all mice infected with RadLV-Rs (five mice in each group) and in several independent experiments.*

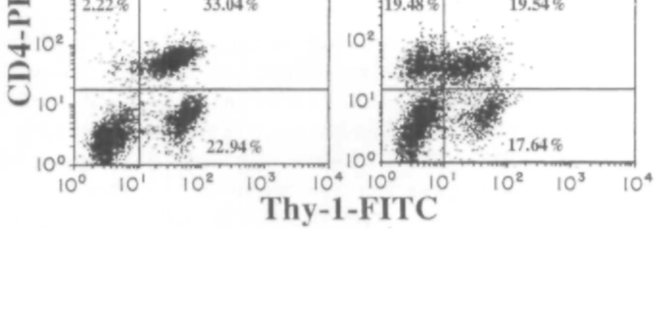

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Fig. 2. *Changes in the frequency of CD4$^+$ cells lacking Thy-1 during the course of RadLV-Rs infection. These results represent the mean frequency (±SD) of CD4$^+$ Thy-1$^-$ cells within the total CD4$^+$ population in (-▲-) LN, (-○-) SP and (-●-) thymus of infected mice (n = 5). No variation was observed in mock-injected controls (not shown).*

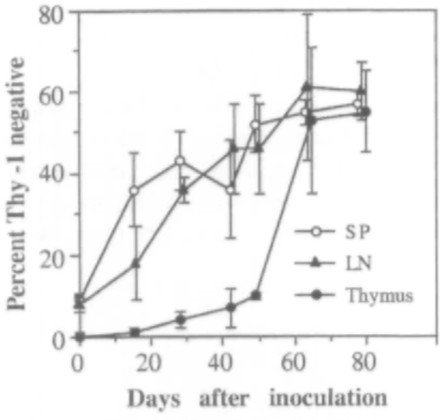
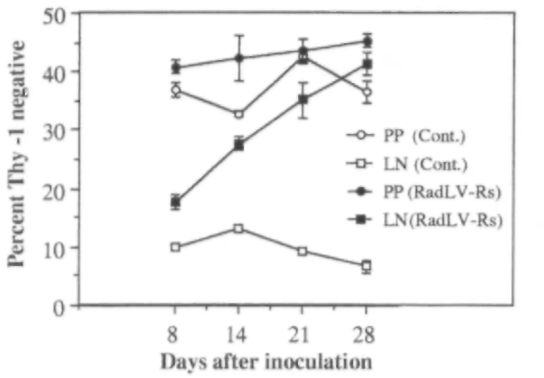


Fig. 3. *CD4$^+$ Thy-1$^-$ T-cells in the Peyer's patches of infected animals. PP and LN lymphocytes were stained with FITC labelled anti-Thy-1.2 and PL-labelled anti-CD4. The results express the proportion of Thy-1$^-$ cells within the CD4$^+$ T-cell population of (-○-) control PP, (-□-) control LN. (-■-) RadLV-Rs infected PP, (-■-) RadLV-Rs infected LN and represent mean±sd from groups of th ree mice.*



In separate experiments, the frequency of CD4$^+$ cells lacking Thy-1 in the Peyer's patches (PP) of RadLV-Rs infected animals and controls was analysed (Fig. 3). Consistent with previous findings [18], normal mice had a high frequency of CD4$^+$ Thy-1$^-$ lymphocytes (around 40% of CD4$^+$ cells). In contrast with observations in SP and LN where the relative frequency of CD4$^+$ lacking Thy-1 increased up to six-fold, there was only a minor increase of this frequency in the PP of infected mice where the proportion of CD4$^+$ T-cells lacking Thy-1 always remained less than 50% after RadLV-Rs inoculation (Fig. 3), A similar picture was found in the liver where 20-30% of CD4$^+$ cells were Thy-1$^-$ in control mice. There was no early increase of this subset in infected mice (unpublished observations), furthermore the fraction of cells with an intermediate expression of TCR *α/β* did not increase in the infected animals (unpublished observations).

Identical expression of CD3 and TCR α/β in Thy-1$^+$ and Thy-1$^-$ T-cells of infected mice. Increased expression of CD44 by CD4$^+$ Thy-1$^-$ T-cells

T-cells lacking Thy-1 have been found with low surface expression of CD3 and TCR *α/β* in mice with the MRL/*lpr* and *gld* mutations [19, 20], so three-colour staining was used to compare the expression of these differentiation markers on CD4$^+$ Thy-1$^+$ and Thy-1$^-$ T-cells from RadLV-Rs infected mice (Fig. 4). No difference was found between both subsets of CD4$^+$ cells in infected mice confirming the belonging of CD4$^+$ Thy-1$^-$ lymphocytes to the T-cell lineage and arguing against an extrathymic differentiation pathway.

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However the CD4$^+$ Thy-1$^-$ subset displayed a particular pattern of CD44 expression. Murine antigen-primed T-lymphocytes can be identified phenotypically by high-level surface expression of CD44 (Pgp-1) [22]. The expression of CD44 was compared in LN CD4$^+$ Thy-1$^-$ and Thy-1$^+$ T cells during the first weeks of the infection (Fig 5). In control animals, the minor CD4$^+$ Thy-1$^-$ subset resembled other CD4$^+$ lymphocytes as to the expression of CD44. On the contrary, most CD4$^+$ Thy-1$^-$ lymphocytes from infected mice expressed a high density of CD44 even in the early stage of the infection when a significant fraction of CD4$^+$ Thy-1$^+$ cells was still CD44^{lo}(Fig. 5).

Fig. 4. *Expression of TCR α/β (A, C, E) and CD3 (B, D, F) by LN CD4$^+$ T-cells (A, B) from a control mouse and CD4$^+$ Thy-1$^+$ (C, D) versus Thy-1$^-$ cells (E, F) from a RadLV-Rs infected mouse (week 6). LN cells were stained with PE-labelled anti-CD4, FITC-labelled anti-Thy-1.2 and biotinylated anti TCR α/β or anti-CD3 revealed with streptavidin-red^{613}. Gates are set on CD4$^+$ cells for the control mouse and on CD4$^+$ Thy-1$^+$ and CD4$^+$ Thy-1$^-$ cells lor the infected mouse. The light histograms are shown as internal negative controls and represent anti-CD3 or anti-TCR α/β reactivity within gated CD4$^+$ Thy-1$^-$ cells (presumed to be mostly non T-cells) from the same mice.*

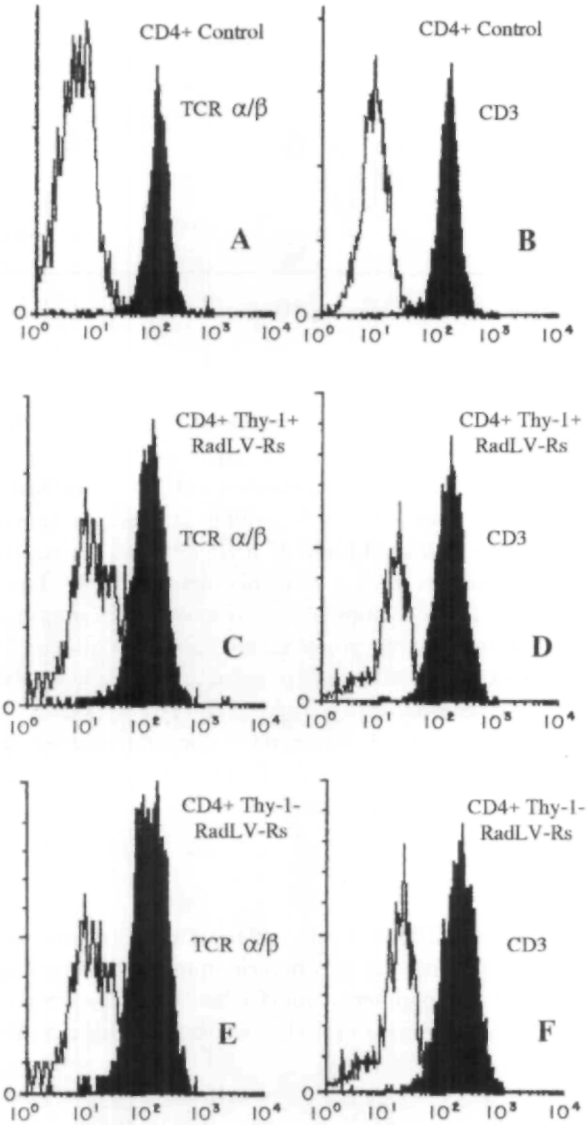
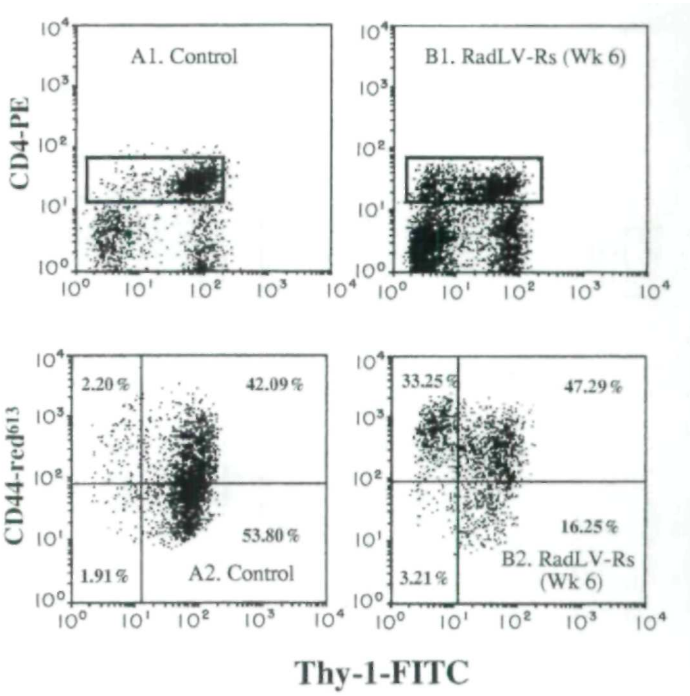


Fig. 5. *High-level surface expression of CD44 by LN CD4$^+$ Thy-1$^-$ T-cells from RadLV-Rs infected mice. LN cells from (A) a control mouse and (B) a RadLV-Rs infected mouse (6 weeks post-infection) were stained with PL-labelled anti-CD4, FITC-labelled anti-Thy-1.2 and biotinylated anti-CD44 revealed with streptavidin-red^{613}. On Thy-1 (FL1) versus CD4 (FL2) dot plots (A1, B1), a gate is set on CD4$^+$ cells. Gated dot plots (i.e. CD4$^+$ T-cells) of Thy-1 (FL1) versus CD44 (FL3) are displayed in A2 and B2. In the infected mouse, the proportion of CD4$^+$ Thy-1$^+$ cells with high expression of CD44 is 74.43% while it is of 96.65% for CD4$^+$ Thy-1$^-$ cells. In contrast, the level of non-specific binding of an irrelevant Rat IgG$_{2b}$, antibody is similar in both subsets of CD4$^+$ T-cells (not shown). Similar dot plots were obtained with LN cells from all mice infected with RadLV-Rs (live mice in each group) and in several independent experiments.*



Population dynamics in CD4$^+$ Thy-1$^+$ and Thy-1$^-$ T-cells

The faster expansion of CD4$^+$ Thy-1$^-$ compared to CD4$^+$ Thy-1$^+$ T-cells in the LN of infected mice prompted investigation into whether this difference could be ascribed to a higher *in situ* proliferative activity in the Thy-1$^-$ subset from LN. Both CD4$^+$ T-cell subsets from infected mice between week 4 and week 6 after inoculation and controls were sorted by flow cytometry (Table 2). After sorting, nuclei were isolated and DNA was stained with propidium iodide. In control mice, the frequency of LN CD4$^+$ cells in S + G$_2$M phases of the cycle (referred here as proliferating cells) was always less than 5%. In infected mice, the proliferative activity displayed by LN CD4$^+$ T-cells was increased with a frequency of S + G$_2$M cells ranging between 5 and 10% (Table 2). Nevertheless despite their 10-fold higher expansion, CD4$^+$ Thy-1$^-$ T-cells from infected mice did not display an increased proliferative fraction compared to the CD4$^+$ Thy-1$^+$ cells from the same mice (Table 2). Also compared to rule out the existence of a more important cycling pool outside the LN were the proliferative fractions of both CD4$^+$ subsets in LN. SP and PP. For both CD4$^+$ subsets of infected mice, the proliferating fractions were comparable in SP and LN while lower values were found in the PP (Fig. 6). Moreover, cell cycle analysis on unsorted thymocytes failed to reveal an early increase in proliferative fraction after infection (Table 3).

Table 2. *LN CD4$^+$ T-cell subsets in MAIDS. DNA distribution*

	S	G2/M
Controls		
total CD4^f	2.71 ± 1.34%	0.26 ± 0.23%
RadLV-Rs		
CD4$^+$ Thy-1$^+$	7.25 ± 0.52%	0.82 ± 0.41%
CD4$^+$ Thy-1$^-$	6.10 ± 1.56%	0.71 ± 0.14%

The results represent means + SD from three mice between week 4 and week 6 post-inoculation and three controls.

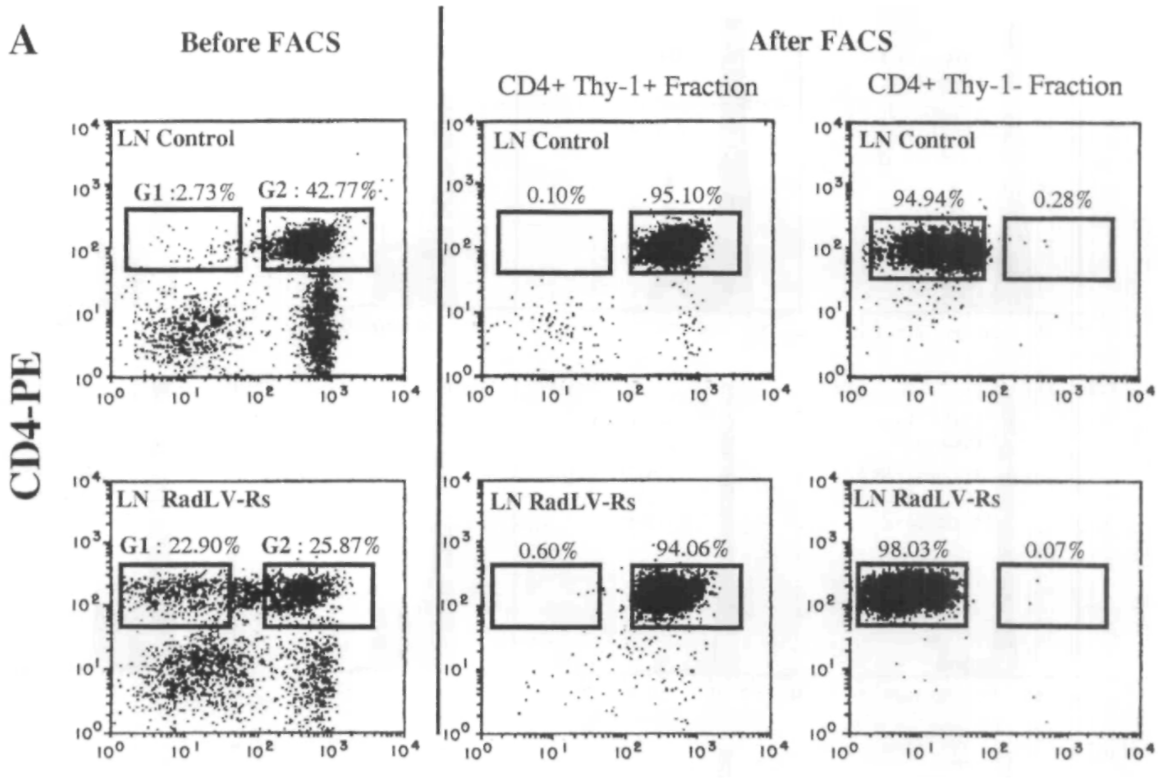
Table 3. *DNA distribution in thymocytes*

	S	G2/M
Controls	I2.62 ± 1.89%	5.50 ± 0.99%
RadLV-Rs	14.5.1 ± 2.25%	3.20 ± 0.72%

The results represent means + SD from groups of three mice (week 4 post-inoculation) and three controls.

Next, it was hypothesized that the Thy-1$^-$ T-cell subset might have an increased life-span which is responsible for their accumulation to the LN of infected animals. To estimate the mean cell life-span, the rates of decay in LN T-cell numbers after two consecutive injections of HU at 7 h-inlerval were studied . This treatment is expected to kill all dividing cells in a few hours. Therefore, cell depletion observed afterwards reflected cell turnover in the absence of replenishment from the cycling pool [26, 29]. Accordingly, 1 h after administration, HU had no effect on the number of CD4$^+$ T-cells in the LN of normal mice (Fig. 7). A significant depletion (around 30%) was observed at 24 h and 72 h after HU administration. In infected mice at week 8 post-inoculation, CD4$^+$ Thy-1$^+$ and CD4$^+$ Thy-1$^-$ populations displayed an important depletion with a maximum around 60% al 72 h. This indicated that for both CD4$^+$ subsets of infected animals, the turnover was increased compared to normal CD4$^+$ Thy-1$^+$ T-cells from non-infected animals. However, no major difference was seen between the depletion patterns of CD4$^+$ Thy-1$^+$ and CD4$^+$ Thy-1$^-$ T-cells of infected mice.

Fig. 6. *DN A distribution in CD4$^+$ Thy-1$^+$ and CD4$^+$ Thy-1$^-$ T-cells in different lymphoid organs of infected mice. SP, LN and PP cells from a mock-injected control and a RadLV-RS infected mouse (6 weeks post-infection) were stained with FITC labelled anti-Thy-1.2 and PE-labelled anti-CD4. CD4$^+$ Thy-1$^+$ and CD4$^+$ Thy-1$^-$ T-cells were sorted by flow cytomelry. (A) shows dot plots and sorting gates for LN cells before and after FACS. Ail dot plots are made with ungated data. The sorting gate set on CD4$^+$ Thy-1$^-$ cells is slightly larger for the control mouse than for the infected animal to increase recovery. Purity is calculated according to the logical gales (see Materials and Methods). Similar sorting gates were used for SP and PP (not shown). (B) Isolated nuclei from the sorted cells were stained with propidium iodide. Data were analysed with the* CELLFIT *software. The histograms were normalized using the same software.*



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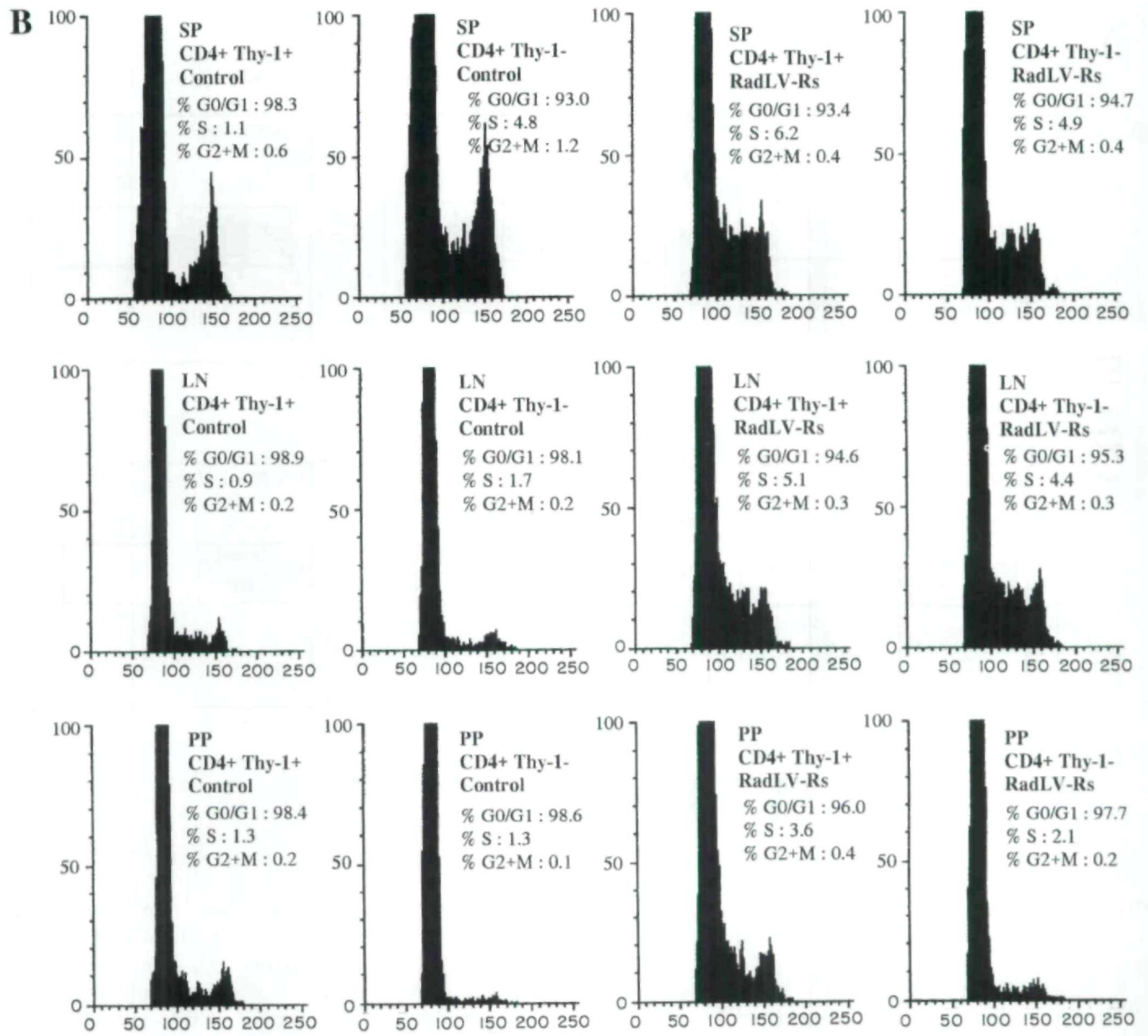
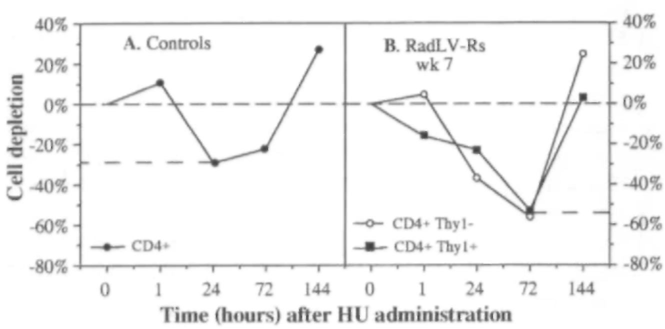


Fig. 7. *Effects of HU on the total number of (A) (-●-) CD4$^+$ T-cells in the LN of control mice and (B) (-○-) CD4 ' Thy-1$^-$ and (-■-) CD4$^+$ Thy-1$^+$ T-cells in the LN RadLV-Rs infected mice (7 weeks post-infection), The results represent the differences between the total numbers of cells ofeach subset in the LN of HU-treated mice and the corresponding numbers in PBS-treated mice at various intervals. The depletions are expressed as a percentage of control values (i.e. total cell numbers in LN of PBS-treated mice). A pool of three animals per experimental group was used. Similar results were observed in three independent experimenls.*



DISCUSSION

The expansion of $CD4^+$ T cells lacking Thy-1 is an intriguing feature of retrovirus-induced murine immunodeficiency syndrome] 15]. The experiments reported here pun ide several new observations with regard to the development of this cell subset in MAIDS.

(1) $CD4^+$ $Thy\text{-}1^-$ T-cells are absent in the thymus of normal mice and display a delayed appearance in the thymus of infected animals compared to peripheral lymphoid organs. Therefore, the expansion of $CD4^+$ $Thy\text{-}1^-$ T-cells in MAIDS is unlikely to result directly from a differentiation event involving immature T-cells of thymic origin.

(2) $CD4^+$ $Thy\text{-}1^-$ T-cells in MAIDS display the same surface density of TCR α/β and CD3 than $CD4^+$ $Thy\text{-}1^+$ T-cells. Furthermore these cells are not amplified in the liver of infected mice. These results contrast with the extrathymic differentiation pathway of T-cells lacking Thy-1 described in other models [20, 31].

(3) The important $CD4^+$ $Thy\text{-}1^-$ T-cell population normally present in the PP is only weakly modified during MAIDS. Furthermore, the proportion of cycling cells is not higher in $CD4^+$ $Thy\text{-}1^-$ than in $CD4^+$ $Thy\text{-}1^+$ T-cells whatever the organ considered (PP, LN and SP). This could mean that the preferential amplification of the $CD4^+$ $Thy\text{-}1^-$phenotype does not result from the expansion of the preexisting $Thy\text{-}1^-$ pool due to a higher sensitivity to retrovirus-dependent mitogenic signals.

(4) Compared to $Thy\text{-}1^+$ T cells, $CD4^+$ $Thy\text{-}1^-$ T-cells uniformly present a primed/memory $CD44^{hi}$ phenotype even in the early stages of the infection.

(5) $CD4^+$ $Thy\text{-}1^-$ and $Thy\text{-}1^+$ T-cells of infected animals display similar persistence after arrest of cell production by HU treatment, indicating comparable lifespans in both subsets. Together with the similarity in proliferating fractions and the fact that invariably, the same ratio is reached between $CD4^+$ $Thy\text{-}1^-$ and $Thy\text{-}1^+$ T-cells in all organs tested, these data indicate close relationships between both $CD4^+$ subsets in MAIDS.

The results suggest that at least part of the expansion of the CD4' $Thy\text{-}1^-$ phenotype is due to differentiation of $Thy\text{-}1^+$ cells. Furthermore, the uniform $CD44^{hi}$ phenotype of $CD4^+$ $Thy\text{-}1^-$ cells in MAIDS indicates that such a differentiation could be associated with TCR triggering. In non-infected mice, it is very likely that most $CD4^+$ $Thy\text{-}1^-$ cells derive from $CD4^+$ $Thy\text{-}1^+$ T-cells rather than from an extrathymic differentiation pathway since $CD4^+$ $Thy\text{-}1^-$ T-cells are absent in the PP and in the other lymphoid organs of nude mice [18]. Furthermore a large proportion of PP $CD4^+$ $Thy\text{-}1^+$ T cells lose Thy-1 expression after *in vitro* activation with anti-CD3 MoAb [18]. In the study described here several attempts to demonstrate the spontaneous loss of Thy-1 *in vitro* by sorted $Thy\text{-}1^+$ T-cells from infected animals were unsuccessful (data not shown). This may be due to the poor survival of the lymphocytes from infected animals in cultures longer than 48-72 h.

The molecular mechanisms responsible for the loss of Thy-1 expression in MAIDS have yet to be defined. Modulation of Thy-1 epitopes due to modified glycosylation patterns could be involved since glycosylation of Thy-1 has been shown to change with T-cell differentiation [32]. The lack of Thy-1 expression might result also from activation of phosphalidylinositol-specific phosopholipase C or defective synthesis of GPI-linked membrane proteins. However, this is unlikely since Holmes *et al.* found a normal expression of Ly-6A/E, another GPI-linked surface molecule on $CD4^+$ $Thy\text{-}1^-$ T-cells in MAIDS [16], The uniform memory/activated phenotype of $CD4^+$ $Thy\text{-}1^-$ T-celts in MAIDS suggests a possible relationship with sustained polyclonal T-cell activation observed in the syndrome. Interestingly. $CD4^+$ $Thy\text{-}1^-$ T-cells have been observed in the LN germinal centers of autoimmune NZB mice [33]. It remains to be established whether any chronic stimulation of T-cells *in vivo* can result in the development of this phenotype or if abnormal signals occurring in MAIDS are specifically involved. Kanagawa *et al.* showed recently that the Du5H-encoded superantigen expressed on infected B-cells elicits different TCR-mediated signals than nominal antigens [34].

Appearance of immunodeficiency in RadLV-Rs infected mice occurred simultaneously with the emergence of the $CD4^+$ $Thy\text{-}1^-$ phenotype (data not shown). Since Thy-1 is associated with the $p60^{fyn}$ tyrosine kinase on T-cells [17], it is possible that the loss of Thy-1 expression is associated with functional abnormalities of $CD4^+$ T-cells. It has been proposed that human CD7 is a functional homologue of murine Thy-1 135]. The emergence of activated $CD4^+$ $CD57^+$ T-cells lacking CD7 expression has been described recently in patients infected with HIV [36]. Interestingly, these cells are refractor) to TCR stimulation [36] and could participate in the dysregulation of the T_{H1}-T_{H2} cytokine network in AIDS (M. Debré *et al.* unpublished observations). As similar disorders of the cylokine secretion patterns have been described in MAIDS [13], it is believed that the loss of Thy-1 by $CD4^+$ cells could constitute a useful phenotypic marker for the study of retrovirus-induced functional disorders of T-cell differentiation.

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