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# LD1: a $CD4^-CD8^-$ $TCR\alpha\beta/CD3^+$ peripheral T cell line with helper function for B lymphocytes

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## Abstract

**In order to learn more about the small subset of CD4-CD8- $TCR\alpha\beta/CD3^+$ peripheral T lymphocytes, we firstly characterized at the cellular and molecular levels the CD4-CD8- LD1 cell line isolated from the spleen of an MRL/lpr – lpr mouse. Secondly we studied its functional properties. LD1 cells are Thy1+ CD5+ CD4-CD8- LFA-1+ PgP-1+ and do not bind the T cell precursor-specific antibodies Joro 37-5 or Joro 75. They are negative for IgM, B-220, BP-1, J11d, Lyb8, Ia, F4/80, BP-2, and Mac-1 surface markers. LD1 cells have deleted the TCRδ locus, have rearrangements at the TCRγ gene cluster (i.e. a $V_\gamma1-J_\gamma1-C_\gamma1$ and a $V_\gamma4.3-J_\gamma4-C_\gamma4$) and have two rearrangements of the TCRβ gene cluster (i.e. a $D_\beta1-J_\beta1$ and $V-D-J_\beta2$). LD1 cells produce normal sized RNA transcripts from TCRα and β genes and lower levels of γ-mRNA. These cells bind CD3- and pan-TCRβ-specific antibodies as determined by FM analysis. We conclude that LD1 cells bear a TCRαβ/CD3 type of receptor complex. LD1 cells fail both *in vivo* and *in vitro* to differentiate into CD4+ or CD8+ cells. These cells help B lymphocytes to mature into antibody-secreting cells, secrete IL-3 and IL-6 but not IL-2, IL-4, or IL-5, and exert no detectable cytolytic activity. These results together with recent reports of antigen-specific $CD4^-CD8^-$ $TCR\alpha\beta/CD3^+$ cytotoxic T cell lines show that the $CD4^-CD8^-$ $TCR\alpha\beta/CD3^+$ subset comprises functionally competent helper and cytotoxic T lymphocytes and thereby argue for their potential to participate in immune responses. Our results also suggest that cells like LD1 represent terminally differentiated T lymphocytes rather than cells with precursor potential for $CD4^+$ or $CD8^+$ $TCR\alpha\beta/CD3^+$ T lymphocytes.**

## Introduction

A CD4-CD8- CD3+ subset of thymocytes was first identified in man (1). Subsequently, the existence of CD4-CD8- TCRαβ/CD3+ thymocytes in normal mice was documented (2 – 4). These mouse thymocytes were found to appear late in ontogeny (3), the time when CD4-CD8- TCRαβ/CD3+ cells appear in the human thymus has not been defined. *In vitro*, CD4-CD8- TCRαβ/CD3+ cells can develop from CD4-CD8- TCR/CD3- PRO-T lymphocyte clones without passing through a CD4+CD8+ stage (5). Recently it was shown that at least some CD4-CD8- TCRαβ/CD3+ cells may leave the thymus and migrate to peripheral lymphoid organs (6). In spite of all this knowledge, there are still several critical aspects of this subset of T cells that remain undefined. Thus, we still lack information on (i) the functional properties of these cells; (ii) whether or not they can develop into CD4+ or CD8+ T cells; (iii) whether or not they are subjected to thymic positive and negative selection processes in a similar fashion to CD4+CD8- and CD4-CD8+ TCRαβ/CD3+ lymphocytes; (iv) whether they recognize antigen in an MHC-restricted manner like the other two major TCRαβ/CD3+ T cell populations do; and (v) whether they can also develop extrathymically.

Mice carrying the Lpr or the Gld mutations develop a lymphoproliferative disorder due predominantly to a massive accumulation of CD4-CD8- TCRαβ/CD3+ lymphocytes (7,8). It is unclear, however, whether the accumulation of these cells in lymphoid organs of these mutant mice is due to an over-production and consequent progressive accumulation in lymphoid tissues, to an increased life span of peripheral CD4-CD8- TCRαβ/CD3+ cells, or to both. Patients suffering from systemic lupus erythematosus (SLE) were recently reported

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to have increased numbers of CD4-CD8- TCRαβ/CD3+ lymphocytes (9). These observations in man and in mice suggest that CD4-CD8- TCRαβ/CD3+ lymphocytes could be involved in the physiopathogeny of some autoimmune diseases and lymphoproliferative disorders.

Several years ago we described the establishment in culture of the LD1 CD4-CD8- Thy1+ cell line which was obtained from the spleen of an MRL/Lpr–Lpr mouse (10). We have now characterized further these cells and found that they carry on the cell membrane a TCRαβ/CD3 type of receptor complex. We then used this CD4-CD8- TCRαβ/CD3+ cell line both to test whether CD4-CD8- TCRαβ/CD3+ lymphocytes can generate CD4+ or CD8+ T cells and to gain insight on the functional properties of this T cell subset

## Methods

### *Mice*

C57BL/6 Nu/Nu mice (5- to 7-week-old females) were from Bomholgard (Denmark). AKR/J mice (8- to 12-week-old male and female) were from our animal facilities

### *Cell preparations*

Spleen and thymus cell suspensions were prepared free of erythrocytes as described (5,10). Cells were washed and resuspended in culture medium [Iscove's modified Dulbecco medium + FCS (5%) + 2-mercaptoethanol (2ME; $5 \times 10^{-5}$ M) + L-glutamine (2 mM) + gentamycin (50 μg/ml)] at the desired concentrations.

### *Cell lines*

The LD1 cell line was obtained from the spleen of an MRL/Lpr–Lpr mouse and established in culture using culture medium supplemented with WEHI-3 supernatants (10). These cells were subsequently found to grow in culture medium without exogenous growth factors (10). LD1 cells are not malignant cells in that they do not form tumors in Nu/Nu mice during 12–16 weeks of observation (R.P., unpublished observations). LD1 cells expressing the highest levels of CD3 were isolated by cell sorter using a FACS 440 instrument as described (11). They were subsequently expanded and were used in all experiments described here. LD1 cells are propagated in tissue culture flasks by changing them in fresh culture medium (~$2 \times 10^5$ cells/ml) every 2–3 days.

The 2.19 CD4+CD8- TCRαβ/CD3+ T helper line (12), the marrow PRO-T clone C4-77/3 (13), the fetal thymocyte clone FTH5 (14), the IL5-responsive B13 cell line (15), the IL6-sensitive hybridoma B45 (provided to us by A. Rolink, Basel Institute for Immunology, Basel), and the ET thymic epithelial clone (5), were also used.

### *Monoclonal antibodies*

mAbs against the following surface antigens were used: B-220 (hybridoma 14.8), Lyb8 (hybridoma Cy 341.2), BP-1, J11d, PgP-1 (hybridoma I42/5), BP-2, F4/80, Mac-1 (hybridoma M1/70), Ia (hybridoma M5/114), IL5R (hybridoma R52.120), IL2R (hybridoma 7D4), IL3R (hybridoma CC11), CD4 (hybridoma gk1.5), CD3 (hybridoma 500A2), pan TCR β chain (hybridoma H57-597), LFA-1 (hybridoma FD441-8), and T cell precursors (hybridomas Joro 37-5, Joro 75). The reports describing the development and characterization of all of these mAbs are listed elsewhere (5,11,13–15,17). Fluorescein isothiocyanate (FITC)- and biotin-conjugated anti-Thy1.2 or LyT2 (CD8) and PE-L3T4 (CD4) were from Becton Dickinson Phycoerythrin (PE)- or FITC-labeled goat anti-hamster IgG, FITC anti-mouse Ig, FITC anti-rat IgG, and PE–streptavidin were from Southern Biotechnology Associates. FITC-conjugated anti-rat IgG1, IgG2a, IgG2b, and IgM were from Binding Site Ltd and FITC–streptavidin was from Amersham.

### *Immunofluorescence staining and flow fluorometry (FM) analysis*

Single and two color stainings were performed as detailed elsewhere (5) and FM analysis was carried out with a FACScan instrument (Becton Dickinson). The data collected from 1–$2 \times 10^4$ cells were analyzed with Consort 30 software (5).

### *Functional assays*

*Differentiation of LD1 cells.* LD1 cells suspended in PBS were injected ($10^6$ cells/10 μl/lobe) into the thymus of sublethally irradiated (600 rads of γ-rays) H-2-compatible Thy1 congenic AKR/J mice as described (16,17) Two and 4 weeks later, the thymuses were obtained, cell suspensions were prepared, and the presence of Thy1.2+ CD4+ or Thy1-2+ CD8+ donor-derived cells was determined by two-color FM analysis.

In another set of experiments, LD1 cells ($10^5$ cells/well) were cultured with monolayers of the ET thymic epithelial clone in six-well Costar plates as detailed elsewhere (5). As a positive control, FTH5 PRO-T lymphocytes (14) were co-cultured with ET thymic epithelial cells (5). Ten days later, the lymphoid cells were harvested and the presence of CD4+, CD8+, and CD3+ cells was determined by two-color FM analysis. LD1 cells ($0.5 \times 10^6$ cells/ml of culture medium) were exposed to several doses of 5-azacytidine (Sigma Chemicals Co., final concentrations 1, 10, 20, 40, and 50 μg/ml) at 37°C for 3–5 days in cultures grown in tissue culture flasks (AS Nunc, Denmark). The cells were harvested, washed, and assayed for expression of surface CD4 or CD8 by single-color FM analysis.

In all experiments, thymocytes from normal AKR mice were used as positive controls in FM analysis.

*Production of cytokines.* LD1 cells ($10^6$ cells/ml) were cultured in medium alone or in culture medium containing phorbol myristate acetate (PMA, 10 ng/ml) and ionomycin (500 ng/ml) at 37°C for 48 h. The supernatants were collected, filtered (0.2 μm Acrodisc filters) and assayed for cytokine activity. The presence of interleukin 2 (IL-2), IL-3, IL-4, IL-5, or IL-6 activity in the supernatants was determined as detailed elsewhere (15,18) using the following target cells: FTH5 (IL-2 and IL-4), C4-77/3 (IL-3), B13 (IL-5), and B45 (IL-6). As positive controls in these assays, we used recombinant interleukins (19) at a concentration of 20 U/ml. Because B13 cells used to detect IL-5 also proliferate in IL-3 (15), the presence of IL-5 in the supernatants tested was assessed by performing the assays in the presence or absence of the IL-5R-specific mAb R52.120 (15) (final concentration 25 μg/ml). The presence of IL-3 activity in the supernatants was determined by using C4-77/3 cells, which respond to IL-3 but not to IL-5, and B13 cells, which respond to both IL-5 and IL-3. FTH5 cells proliferate in either IL-2 or IL-4, but not IL-3, IL-5, or IL-6. Therefore, they were used to detect IL-2/IL-4 activities.

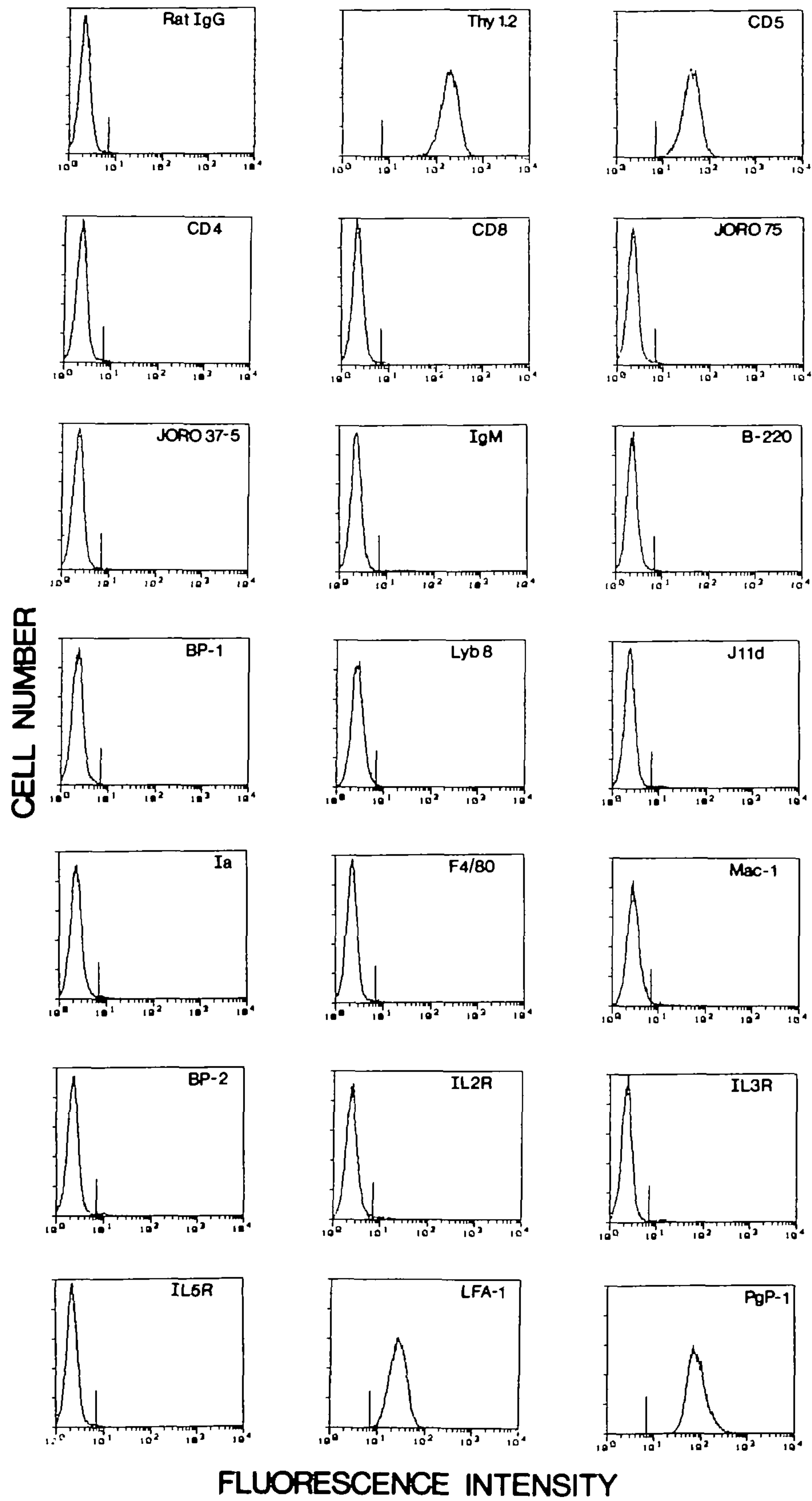


**Fig. 1.** Phenotype of LD1 cells was determined by single-color FM analysis.

Cell proliferation was measured by [$^3$H]thymidine uptake (1 μCi/well, TRA 185 MBq, Amersham) during the last 6 h of a 2 day culture period. The results are given as units/ml of biological activity, where 1 unit is defined as the dilution giving half of the maximum proliferative response.

*Helper and cytotoxic T cell functions.* The capacity of LD1 cells to assist B lymphocytes from spleen of C57BL/6 Nu/Nu mice to mature into antibody-secreting cells was studied in a Concanavalin A (Con A)-facilitated microculture assay as detailed before (10). The FTH5 PRO-T clone and lipopolysacharides (LPS) (50 μg/ml) were included in this assay as negative and positive controls, respectively. The number of antibody (IgM)-secreting cells was determined by the reverse hemolytic plaque assay (20) after 5 days of culture at 37°C. The results are expressed as PFC per culture and are the mean of triplicate cultures.

The capacity of LD1 cells or the cytotoxic 4E7 T cells (21) (positive control) to exert cytotoxic activity was tested in a PHA-facilitated microcytotoxicity assay using as target cells $^{51}$Cr-labeled LPS-stimulated spleen cells or EL-4 T lymphoma cells and were performed for us by H. G. Rammensee as described (21).

*Isolation and analysis of nucleic acids.* DNA and RNA preparation, restriction enzyme digestions, agarose gel electrophoresis, DNA and RNA blotting procedures, probe preparations, hybridization procedures, and autoradiography were performed as described previously (14,22,23).

*DNA probes.* The following probes used were isolated inserts which were $^{32}$P-labeled using a random primed DNA labeling kit (Boehringer Mannheim, Mannheim). The 5′ $D_\delta 1$ genomic probe (600 bp *Hin*dIII – *Hin*dIII fragment), the 5′ $D_\delta 2$ genomic probe (850 bp *Hin*dIII – *Eco*RI fragment) (23), the $J_\gamma 1$ probe (1.2 kb *Ava*I – *Hin*dIII genomic fragment), the $c_\gamma 2$ probe (1.0 kb *Eco*RI – *Hin*dIII genomic fragment) (24), the $c_\gamma 1$ cDNA probe (900 bp *Ava*I – *Eco*RI fragment) (25), the 5′ $D_\beta 1$ probe (1.2 kb *Pst*I – *Acc*I genomic fragment), the $J_\beta 1$ probe (2.0 kb *Pst*I – *Eco*RI genomic fragment), the $J_\delta 1$ probe (400 bp *Pvu*II – *Sau*3A genomic fragment), the $J_\beta 2$ probe (800 bp *Cla*I – *Eco*RI genomic fragment) (26,27), the 4.1 cDNA probe specific for the constant region of the TCR β gene complex (28), and the $C_\alpha$ probe (300 bp *Nco*I – *Ava*II fragment from the constant region of the α chain cDNA clone T1.2) were used.

## Results and discussion

*Phenotype*

The LD1 cell line was phenotypically characterized by immunofluorescence staining and FM analysis using a panel of mAbs reactive with surface molecules expressed by T lymphocytes, B lymphocytes, or myeloid cells (Fig. 1). Among the T cell lineage surface markers tested, LD1 cells were positive for Thy1 and CD5 (Ly1) but negative for CD4, CD8, and the T cell precursor specific markers Joro 37-5 and Joro 75. LD1 cells expressed neither IgM, Ia, B-220, J11d, BP-1, Lyb8 B cell lineage markers nor F4/80, Mac-1, BP-2 myeloid-cell lineage antigens. They carry LFA-1 and PgP-1 adhesion molecules on the cell membrane but no detectable IL-3R, IL-5R, or the p55 chain of the IL-2R complex.

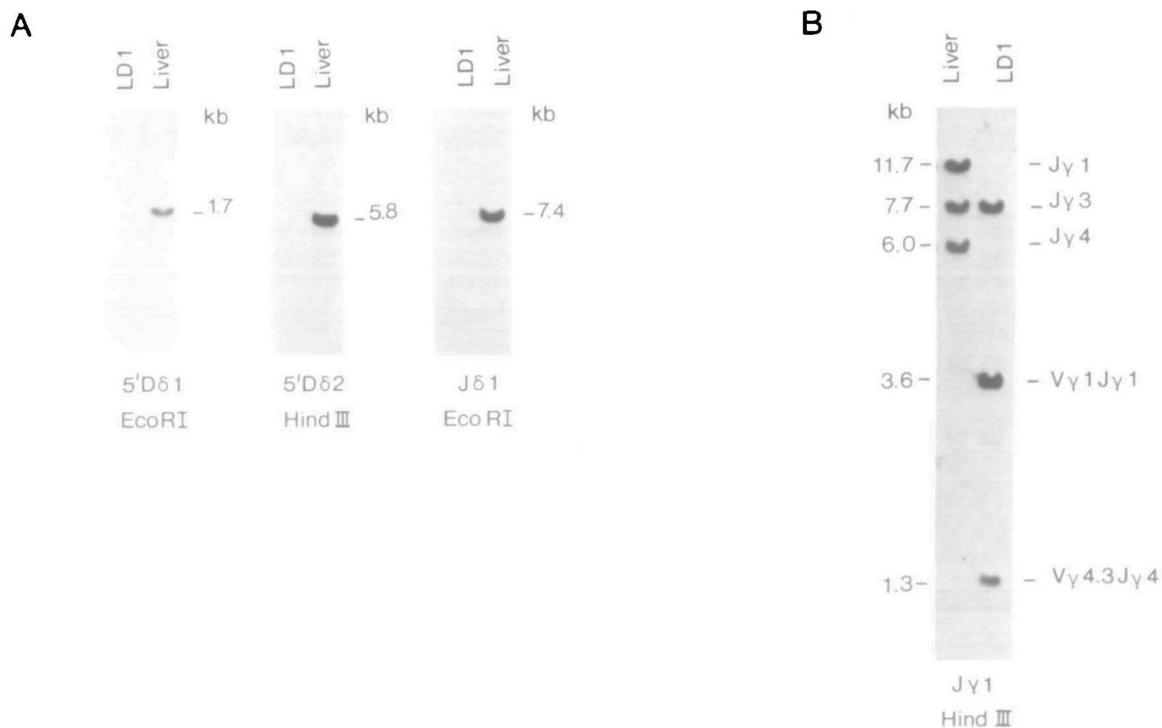


**Fig. 2.** (A) Southern blot analysis of the TCRδ loci in LD1 cells and MRL/lpr – lpr liver (B) Analysis of the TCRγ gene cluster in LD1 cells was carried out by Southern blot analysis. The nomenclature and assignment of germline and rearranged TCRγ gene bands is according to (24).

*LD1 cells express a TCRαβ/CD3 receptor complex*

In order to further characterize the LD1 cell line, we studied by Southern blot analysis the state of the TCRδ, γ, and β genes in these cells. The analysis of *Hin*dIII- or *Eco*RI-digested DNAs from these cells and hybridization with $J_\delta1$-, 5′ $D_\delta1$-, and 5′ $D_\delta2$-specific probes indicated that LD1 cells have deleted the TCRδ locus (Fig. 2A).

LD1 cells had a $V_\gamma1-J_\gamma1-C_\gamma1$ and a $V_\gamma4.3-J_\gamma4-C_\gamma4$, rearrangements in the TCRγ gene cluster (detected by using *Hin*dIII-digested DNA and the $J_\gamma1$ probe) and the γ3 pseudogene in the germline configuration (Fig. 2B). No rearrangement of the γ2 gene was detected using *Eco*RI-digested DNAs and the $C_\gamma2$ probe [nomenclature of TCRγ genes is according to Traunecker *et al.* (26)]. The state of the TCRβ gene was assessed using *Hin*dIII- or *Eco*RI-digested DNAs and probes specific for the $J_\beta1$, $J_\beta2$, 5′ $D_\beta1$, and 5′ $D_\beta2$ chromosomal regions. Figure 3 shows only the results obtained with the *Hin*dIII-digested DNA as the information obtained using *Eco*RI-digested DNA was the same. LD1 cells had a $D_\beta1-J_\beta1$ rearrangement (detected with the $J_\beta1$ and 5′ $D_\beta1$ probes), with $J_\beta2$ and its surroundings in the germline in one chromosome. In the other chromosome there was a deletion of the entire $J_\beta1-C_\beta1$ region and a $V-D-J_\beta2$ rearrangement (detected with the $J_\beta2$ probe). With *Hin*dIII-digested DNA, the 5′ $D_\beta2$ probe only detected the joining of D to $J_\beta2$ and the germline $J_\beta2$ band in the other allele because there is a *Hin*dIII site in the middle of the region comprised by this probe (see Fig. 3 bottom).

Next we assessed by Northern blot analysis the presence of RNA transcripts from the TCRα, β, and γ genes in LD1 cells. As a positive control, RNA from the 2.19 cells, a CD4⁺CD8⁻ TCRαβ/CD3⁺ T helper line (11), was included in these analyses. Figure 4 shows that LD1 cells produce normal sized RNA transcripts from the TCRα and β genes and lower levels of γ-mRNA.

We also studied by FM analysis the expression of TCRαβ/CD3 receptor complex on the cell membrane of LD1 cells using the CD3ε-specific antibody 500A2 (29) and the pan-TCR β chain-specific antibody H57-597 (30). Figure 5 shows that LD1 cells bound both the CD3- and the pan-TCR β chain-specific antibodies. Antibodies against $V_\beta8$ or $V_\beta17a$ gene products did not bind to LD1 cells. Taken together, we conclude that LD1 cells express a TCRαβ/CD3 type of receptor complex.

*Functional potential*

*LD1 cells do not give rise to CD4⁺ or CD8⁺ cells.* The finding that LD1 cells belong to the CD4⁻CD8⁻ TCRαβ/CD3⁺ subset of T cells allowed us to test whether these cells can differentiate further into CD4⁺ or CD8⁺ cells. This was assessed *in vivo* by injecting LD1 cells into the thymus of sublethally irradiated (600 rad) H-2-compatible Thy1 congenic mice (AKR/J, H-2ᵏ, Thy1.1) and assaying the thymuses 2 – 4 weeks later for the presence of Thy1 2⁺ CD4⁺ and Thy1.2⁺ CD8⁺ cells by two-color FM analysis. In all six mice tested, while Thy1.2⁺ cells were detected (range 35.1 – 56%) none of these cells co-expressed CD8 or CD4 surface markers (Table 1).

*In vitro* studies in which LD1 cells were cocultured with the thymic epithelial cells ET [able to induce differentiation of CD4⁻CD8⁻ PRO-T lymphocyte clones into CD4⁺CD8⁻ TCR/CD3⁺ cells (5)] showed that while FTH5 PRO-T cells generated CD4⁺CD8⁻ cells, LD1 cells did not (Fig. 6). 5-Aza-

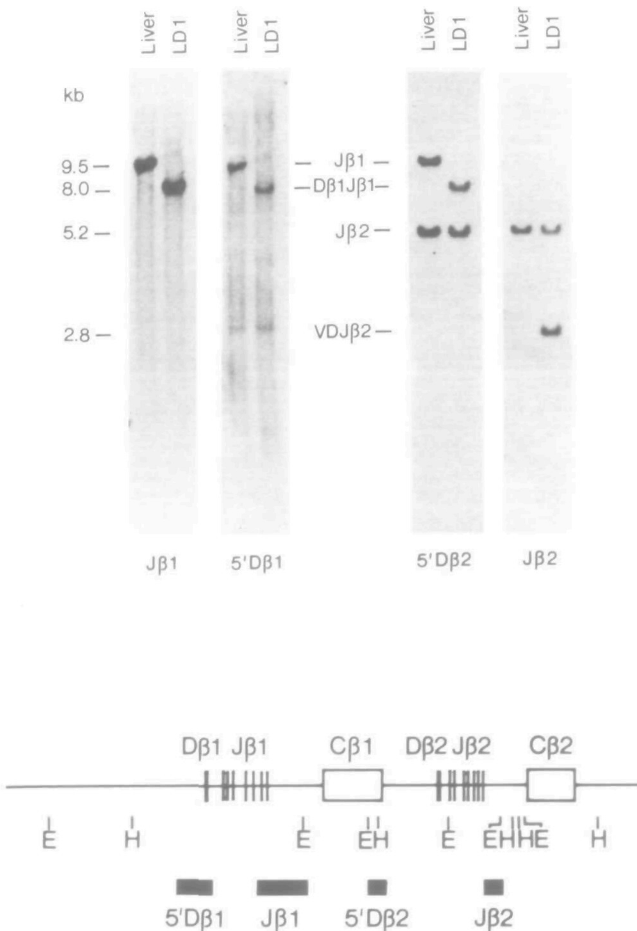


**Fig. 3.** The state of the TCRβ gene cluster in LD1 cells was assessed by Southern blot analysis using *Hin*dIII-digested DNA. Similar information was obtained using *Eco*RI-digested DNA.

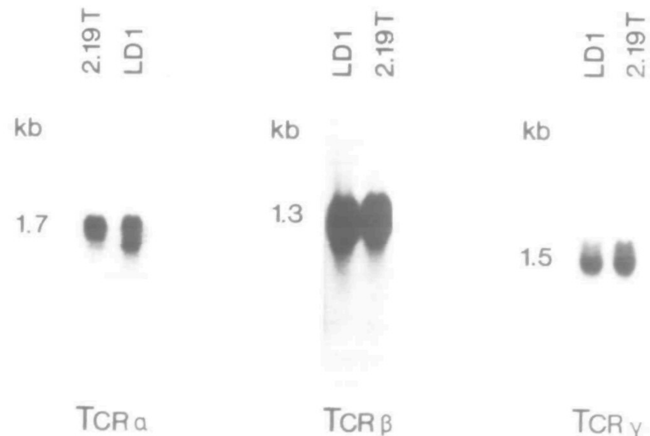


**Fig. 4.** The expression of RNA transcripts from TCRα, β, and γ genes in LD1 cells and 2.19 T cells (positive control) was determined by Northern blot analysis using the constant region-specific probes $C_\alpha$, 4 1 cDNAβ, and $C_\gamma1$ DNA

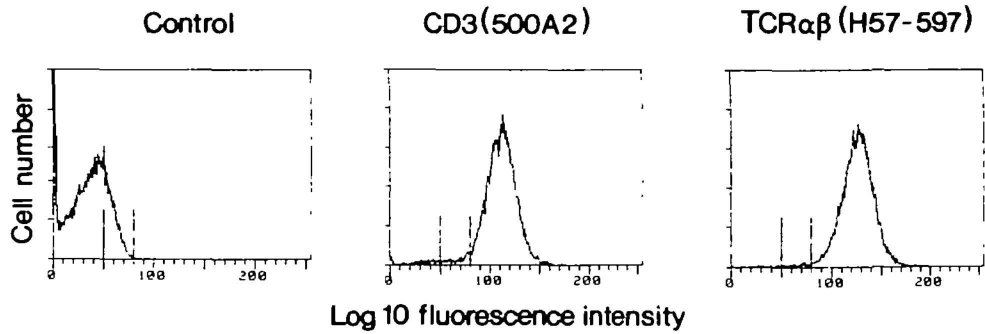


**Fig. 5.** The expression of CD3 and $\alpha\beta$ heterodimers on the cell membrane of LD1 cells was determined by FM analysis with the CD3 $\epsilon$ chain-specific 500 A2 mAb and the pan-TCR $\beta$ chain-specific H57-597 mAb, using as the second-step reagent FITC anti-hamster IgG antibody Control = LD1 cells stained with second-step antibody only.

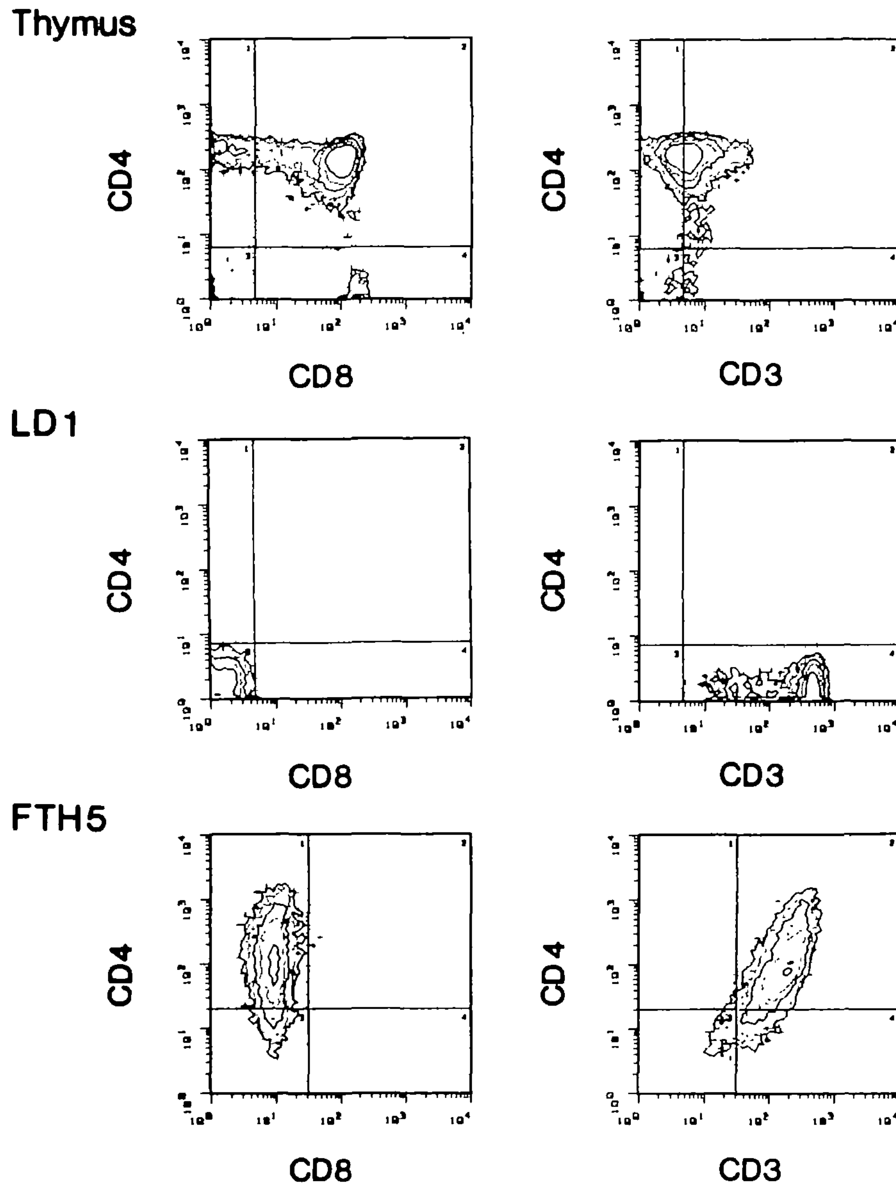


**Fig. 6.** LD1 cells or FTH5 PRO-T lymphocytes were cultured with the ET thymic epithelial cells for 10 days (5). The presence of CD4+, CD8+, and CD3+ cells was determined by two-color FM analysis. ET thymic epithelial cells induce PRO-T cells to generate CD4+CD8− TCRαβ/CD3+, CD4−CD8− TCRαβ/CD3+, and TCRγδ/CD3+ cells but not CD8-bearing cells (5)

**Table 1.** LD1 cells do not give rise *in vivo* to CD4+ or CD8+ cells

| Mouse[a] | % positive cells (FM analysis) | | |
|---|---|---|---|
| | Thy1 2+ | Thy1 2+ CD4+ | Thy1 2+ CD8+ |
| 1 | 35 | <1 | <1 |
| 2 | 42 | <1 | <1 |
| 3 | 56 | <1 | <1 |
| 4 | 38 | <1 | <1 |
| 5 | 40 | <1 | <1 |
| 6 | 39 | <1 | <1 |

[a]LD1 cells were injected into the thymus of sublethally irradiated, H-2-compatible Thy1 congenic AKR/J mice. Two to 4 weeks later, the presence of Thy1.2+ CD4+ and Thy1.2+ CD8+ LD1-derived cells was determined by two-color FM analysis (see Methods for details).

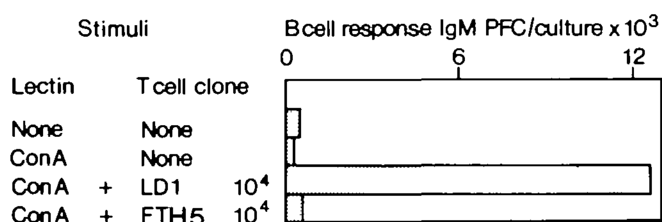


**Fig. 7.** The capacity of LD1 cells to assist B lymphocytes from the spleen of Nu/Nu mice was determined in a Con A-facilitated microculture assay (10). FTH5 PRO-T cells known to lack helper function (14) were used as negative control The number of IgM PFC per culture was determined by the reverse hemolytic Protein A plaque assay.

cytidine, a drug able to induce expression of CD4 or CD8 (13,31), also failed to induce LD1 cells to express CD4 or CD8 at the several concentrations tested (1 – 50 g/ml).

The simplest interpretation of these results is that LD1 cells are CD4-CD8- TCRαβ/CD3+ terminally differentiated lymphocytes rather than cells with precursor potential for CD4+ or CD8+ TCRαβ/CD3+ cells.

*Cytokine production.* The potential of LD1 cells to produce IL-2, IL-3, IL-4, IL-5, and IL-6 was studied by testing supernatants from either (PMA + ionomycin)-stimulated or unstimulated LD1 cells for their growth promoting effect on the respective target cells for the different interleukins studied (see Methods for details). Unstimulated LD1 cells produced little IL-3 and IL-6 activities but no detectable IL-2, IL-4, or IL-5 (Table 2). LD1 cells stimulated by PMA + ionomycin secreted higher levels of IL-3 and IL-6 but no detectable IL-2, IL-4, or IL-5 (Table 2). Previously, it was shown by RNA – RNA *in situ* hybridization that LD1 cells do not synthesize mRNA for IL-4 (14), in agreement with the results obtained here. Thus, LD1 cells can secrete IL-3 and IL-6, but they seem unable to synthesize IL-2, IL-4, and IL-5. It is unclear, at present, whether this pattern of cytokines produced by LD1 cells is a feature of cells generated in Lpr mice, a property of double negative peripheral T cells, or a peculiarity of LD1 cells.

*Helper function for B lymphocytes.* The capacity of LD1 cells to assist B lymphocytes from the spleen of Nu/Nu mice to mature into antibody-secreting cells was studied in a Con A-facilitated assay which detects helper activity for B cells regardless of the

**Table 2.** Secretion of cytokines by LD1 cells (in units/ml)

| LD1 cells treated with | IL-2 | IL-3 | IL-4 | IL-5 | IL-6 |
|---|---|---|---|---|---|
| None | <1 | 17 | <1 | <1 | 12 |
| PMA + ionomycin | <1 | 210 | <1 | <1 | 234 |

Supernatants from LD1 cells cultured in the presence or absence of PMA + ionomycin at 37°C for 48 h were assayed for the interleukins indicated above as detailed in Methods.

specificity of the T cell line studied (10). Negative controls in these experiments were spleen B cells from Nu/Nu mice cultured with either Con A alone or Con A plus the fetal thymocyte clone FTH5 known to lack helper activity (14). Figure 5 shows that LD1 cells efficiently help B lymphocytes to mature into antibody-secreting cells, confirming our previous results obtained with resting B lymphocytes from normal mice (10). We have determined that B cells stimulated by LD1 cells secrete IgM as well as IgG subclasses [IgG1, IgG2a, IgG2b, and IgG3 (10)] LD1 cells tested negative for mycoplasma contamination several times, ruling out the possibility that the helper function for B lymphocytes was due to products of mycoplasma. The helper activity on B lymphocytes of LD1 cells was also documented by other investigators (C. Martinez-A. and A. Coutinho, personal communication). Supernatants from LD1 cells support maturation of B lymphocytes into IgM-secreting cells, suggesting that the helper function for B cells of LD1 cells is, at least partially, due to soluble factors. The soluble factor(s) responsible for the helper activity on B cells remains to be identified, but it must be different from IL-2, IL-4, and IL-5 (Table 2). IL-6 and neuroleukin are potential candidates and experiments to test them are under way.

*Cytotoxic function.* The cytotoxic potential of LD1 cells was assessed in a PHA-facilitated ⁵¹Cr-release cytotoxic assay which detects cytotoxic activity regardless of the specificity of the effector cells. While the CD4-CD8+ cytotoxic 4E7 T cells killed the target cells, LD1 cells did not exhibit this function at the different effector to target cell concentrations tested (data not shown).

*Concluding remarks*

Our results indicate that LD1 cells are CD4-CD8- TCRαβ/CD3+ peripheral T lymphocytes with helper function for B lymphocytes. We failed to obtain *in vivo* or *in vitro* any evidence that LD1 cells have precursor potential to develop into CD4+ or CD8+ lymphocytes. Thus, we favor the view that cells like LD1 represent a separate subpopulation of terminally differentiated mature T lymphocytes.

Although more work is clearly needed to understand the biological meaning for the existence of CD4-CD8- TCRαβ/CD3+ lymphocytes, the finding that cells like LD1 can provide helper function for B lymphocytes (9,10, this study), and the recent reports describing antigen-specific CD4-CD8- TCRαβ/CD3+ cytolytic T cell lines (32,33) argue that at least some CD4-CD8- TCRαβ/CD3+ cells are functionally competent and, thereby, with potential to actively participate in immune responses. A recent study reported increased numbers of CD4-CD8- TCRαβ/CD3+ lymphocytes in patients suffering from SLE, a multisystemic autoimmune disease (9). In order to be able to understand how and if CD4-CD8- TCRαβ/CD3+

lymphocytes participate in the physiopathogeny of autoimmune diseases we must first determine whether or not these cells are subjected to positive and negative selection in the thymus in a similar fashion to CD4+ and CD8+ TCRαβ/CD3+ thymocytes (34), whether or not CD4-CD8- TCRαβ/CD3+ cells can also develop extrathymically, and if the requirements for antigen-specific activation of these cells are similar to or different from those for the other two major subsets of TCRαβ/CD3+ lymphocytes.

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**Abbreviations**

| | |
|---|---|
| Con A | concanavalin A |
| FITC | fluorescein isothiocyanate |
| FM | flow fluorometry |
| IL | interleukin |
| LPS | lipopolysaccharide |
| 2ME | 2-mercaptoethanol |
| PE | phycoerythrin |
| PMA | phorbol myristate acetate |
| SLE | systemic lupus erythematosus |

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