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HLA DR

Lamina Propria-like T
CD2 CD3

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**Phenotypic Characteristics and CD2- and CD3-mediated Signal Transduction
in HLA DR Positive and Negative Subsets of Lamina Propria-like T-cells****Won Ho Kim, M.D., Hyun Soo Kim, M.D., Chung Ryul Lee, M.D.,
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Background/Aims: Peripheral blood T-cells co-cultured with Daudi (a irradiated B-cell line) and interleukin (IL)-2 attain a CD2 dominant cytokine secretion pattern and show tyrosine phosphorylation profile identical to freshly isolated lamina propria (LP) T-cells. Since the phenotypic characteristics and signal transduction pathway of these LP-like T-cells are largely unknown yet, we have explored the expression pattern of T-cell markers and CD2 ligation-induced tyrosine phosphorylation profile of LP-like T-cells and their HLA DR+ and DR- subsets. **Methods:** The LP-like T-cells were sorted into DR+ and DR- subsets by magnetic sorting. Surface markers were measured by flow cytometry. Tyrosine phosphorylation profile was investigated by Western blot. **Results:** The LP-like T-cells consisted of two distinct populations: approximately 55% of the cells express both HLA DR and IL-2R (DR+ LP-like T-cells) and the remainder express neither HLA DR nor IL-2R (DR- LP-like T-cells). Approximately 80% of DR+ LP-like T-cells were memory cells expressing CD45RO and 2/3 of them were activated T-cells expressing CD69, CD71 and CD30. In contrast, 50% of DR- LP-like T-cells expressed CD45RO and only 10-20% of them expressed activation markers. Tyrosine phosphorylation of p72 protein after CD2 ligation was identified only in DR- LP-like T-cells. **Conclusions:** The LP-like T-cells consist of two distinct subpopulation in terms of phenotype and tyrosine phosphorylation pattern according to CD2 ligation. (**Kor J Gastroenterol 1999;34:609 - 618**)

Key Words: Lamina propria T-cells, LP-like T-cells, Phenotype, CD2, Tyrosine phosphorylation

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, IgA
 . LP
 (LP lymphocyte; LPL) 40-90% T T
 /B
 10% .6 LP T - B
 가 LP T
 T (Leu3+, Leu8-)가
 . LP T T
 (CD45RO+)
 interleukin-2 receptor a chain (IL-2Ra), transferrin
 , 4F2 MHC class II 가
 가 . LP T
 가
 (controlled inflamma-
 tion).7 LP T T
 CD3 ligation
 IL-2 가 CD2 ligation
 IL-4
 .7 LP T ,
 LP T 가 , LP T
 CD2
 (effective) (localized) T ,8,11
 (controlled) TCR/CD3 가 ino-
 .45 sitol phospholipid (InsP) protein tyrosine
 Peyer's patch (PP) kinase (PTK) . TCR
 가 IgA T
 (commit) (inductive site) lamina CD2, CD28, CD27, CD40L
 propria (LP) T CD3
 sIgA T .12,13
 (effector site) T leukocyte function
 .1 T antigen-3 (LFA-3, CD58) CD2
 가 InsP PTK .14-18 LP
 , (intraepithelial) T T
 (T-cell receptor, TCR) CD8+ T
 LP CD4+ T
 B IP3 가 가 .19 LP T

가

.12 , tight junction, lysozyme, lactoferrin, amylase

secretory IgA (sIgA)가 , sIgA 1 kg 40 mg IgG .

(lamina propria; LP) 80% 10%가 gut-associated lymphoid tissue (GALT) 가

.3 (immune tolerance) LP T 가 (selective) CD2

(effective) (controlled) (localized) T .8,11 TCR/CD3 가 ino- sitol phospholipid (InsP) protein tyrosine kinase (PTK) . TCR

가 IgA T (commit) (inductive site) lamina CD2, CD28, CD27, CD40L propria (LP) T CD3 sIgA T .12,13 (effector site) T leukocyte function

.1 T antigen-3 (LFA-3, CD58) CD2 가 InsP PTK .14-18 LP , (intraepithelial) T T (T-cell receptor, TCR) CD8+ T LP CD4+ T B IP3 가 가 .19 LP T

CD3 ligation tyro-
 sine (p72) CD2 ligation CD2
 ligation
 .20
 LP T 가
 LP T 가
 T B Daudi IL-2 가
 T CD3
 CD2 CD28 co-
 ligation CD2 ligation
 , LP T
 LP-like T
 .20 LP-like T
 CD2 CD3 ligation tyrosine phosphorylation
 profile LP T
 .
 1. LP-like T
 1:1 Ficoll-hypaque (1.077
 g/mL; Pharmacia Fine Chemicals)
 (density gradient centrifugation; 500 g, 30
) .21
 Hank's balanced salt solution (HBSS)
 RPMI 1640 (Irvine Scientific, Santa Ana, CA)
 5% (Fetal calf serum; Irvine Scientific)
 가 Petri dish 37 ,
 5% CO2 1
 가 100 × 10⁶ mL
 column 37 , 5% CO2
 1
 B 22 T T
 (flow cytometry)
 95% CD3+
 T 10⁶ mL
 3,000 rad 2 × 10⁵ mL

Daudi 10 U/mL recombinant human interleukin-
 2 (rhIL-2; R&D Systems, Minneapolis, MN) 가
 5 37 , 5% CO2 LP-like
 T .20 Daudi
 magnetic sorting CD19+
 .
 2. Magnetic cell sorting DR+
 DR- LP-like T
 Magnetic cell sorting LP-like T
 107 mL 0.25 µg/
 106 HLA-DR (L243) 4
 30 .
 G (goat anti-mouse IgG)
 microbead (Miltenyi Biotec Inc., Sunnyvale, CA)
 107 20 µL 가 4 15
 . MACS (Miltenyi Biotec Inc.)
 sorting .23 LP-like T
 CD19+ CD20+
 가 1% .
 3. Flow cytometry LP-like
 T
 2 × 10⁵ 12 × 75
 mm 2% FCS 0.1% sodium azide
 phosphate buffered saline (PBS-FCS) 100 µL
 4
 20 . FITC가
 1:200
 G 100 µL 가 20 .
 PBS-FCS 3 100 µg/mL mouse
 IgG PBS-FCS 100 µL
 PE가 가
 20 . PE FITC가
 .
 PBS-FCS 1% paraformaldehyde
 가 PBS . Becton-
 Dickinson FACScan
 LYSIS II .24 LP-like
 T

CD45RO CD45RA , CD2 CD3
 CD69 CD71 (transferrin receptor) T
 CD30 .257 DR+ DR- LP-like T

4. Phosphotyrosine Western blot
 5 × 10⁶ mL T 1:50
 CD2 (T11) 10 μL CD3
 (OKT 2 mg/mL) 10 mL 가 . 37 2
 가 10 μg/mL
 가 2 . 4 4,500 rpm 5

2.5 mM EDTA, 2% sodium dodesyl sulfate (SDS),
 20% 2-mercaptapurine (2-ME), 10% glycerol, 0.1 M
 Tris (pH 6.8) bromophenol blue
 (sample buffer) 50 mL 5 4
 , 8,000 rpm
 SDS PAGE BioBlot
 membrane (Costar) (transfer)
 Tris-buffered saline Tween (TBST) 5%
 non-fat milk (blocking) (mouse
 anti-human phosphotyrosine antibody, Upstate Bio-
 technology) 가 4
 TBS-T 3 10,000
 (horse radish peroxidase conjugated sheep anti-
 mouse Ig, Amersham Life Science) 1
 enhanced chemiluminescence (ECL,
 Amersham)

LP-like T
 HLA DR IL-2R
 LP-like T HLA DR
 IL-2R
 (Fig. 1), LP-
 like T HLA DR IL-2R
 DR+ LP-like T ,
 DR-LP-like T . HLA DR
 IL-2R DR+ LP-like T
 forward scatter 가 DR- LP- like T

(two color flowcytometry) DR+
 LP-like T 80% CD45RO
 , 2/3 CD69, CD71 CD30
 , DR- LP-like T
 , 10-20%
 (Fig. 2).
 Sorting LP-like T
 DR+ DR- LP-like T CD2 CD3 ligation
 protein tyrosine phosphorylation profile Western
 blot . Sorting LP-like T
 CD2 CD3 superantigen
 staphylococcal enterotoxin B (SEB)
 10 phosphotyrosine immunoblot
 SEB CD3

Fig. 1. Phenotypic characteristics of LP-like T-cells. LP-like T-cells were stained with FITC-conjugated anti-HLA DR and PE-conjugated anti-IL-2R monoclonal antibodies then analyzed by two color flow cytometry after gating with cell size. LP-like T-cells consisted of two distinct populations; approximately 55% of cells expressing both HLA DR as well as IL-2R (DR+ LP-like T-cells) and the remainder expressing neither HLA DR nor IL-2R (DR-LP-like T-cells).

CD3 ligation tyrosine 72 kDa 가 CD2 ligation DR+ DR- LP-like T (Fig. 3).

Fig. 2. Phenotypic characteristics of DR+ and DR- subsets of LP-like T-cells. DR+ and DR- subsets of LP-like T-cells were magnetically sorted and their expression of CD45RO, CD69, CD71 and CD30 were analyzed by two color flow cytometry. Approximately 80% of DR+ LP-like T-cells were memory cells expressing CD45RO and 2/3 were activated T-cells expressing CD69, CD71 and CD30. In contrast, 50% of DR- LP-like T-cells expressed CD45RO and only 10-20% expressed T-cell activation markers.

Fig. 3. CD2 or CD3 ligation-mediated tyrosine phosphorylation profile of LP-like T-cells. LP-like T-cells were stimulated with CD2 or CD3 ligation and staphylococcal enterotoxin B (SEB) then immunoblot was performed using anti-phosphotyrosine antibody. Immunoblotting revealed inducible 72 kDa protein (p72) phosphorylation during CD2, but not CD3, ligation indicating that postreceptor pathways in LP-like T-cells may be adapted specifically to facilitate CD2-mediated cytokine secretion.

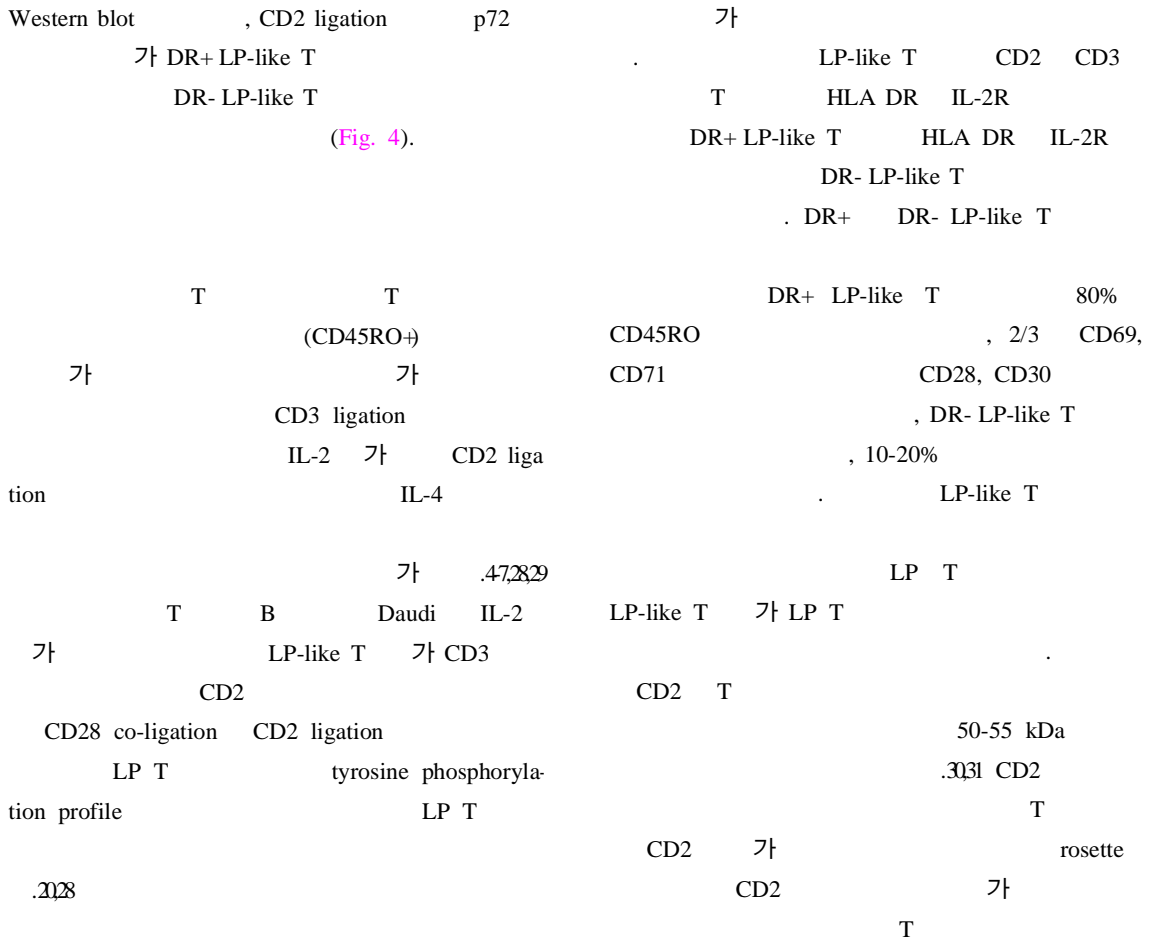


Fig. 4. Tyrosine phosphorylation profile of DR+ and DR- subsets of LP-like T-cells. DR+ and DR- subsets of LP-like T-cells were magnetically sorted and their phosphorylation profile were analyzed by immunoblotting. CD2 ligation-mediated tyrosine phosphorylation of p72 was observed in DR- LP-like T-cells but not in DR+ LP-like T-cells.

CD2 ligand CD3 ligation 72
 CD58 (LFA-3) T tyrosine 가 CD2 ligation
 TCR/CD3 , LP-like T DR+ LP-
 T 가 T , like T CD2 ligation p72
 , T DR- LP-like T
 ,134 T (apoptosis) .
 LP T 가 T 가
 CD2 TCR/CD3 CD2
 .153 CD2 (domain) CD3
 protein tyrosine kinase (PTK) src-homology 3 LP T LP-like T
 (SH3) domain proline DR+ LP-like T DR- LP-like T
 CD2 가 PTK CD2 CD3 ligation tyrosine phosphorylation pro-
 .31 Jurkat CD2 file
 CD3 LP T
 T PTK ZAP- LP T
 70 가 ,34 -chain . CD2 ligation
 immune receptor tyrosine-based activation (ITAM) tyrosine p72
 motif가 T CD2 가
 .35 T
 CD3 CD2 가 .
 cAMP-response element binding protein
 (CREB) 가 CD2
 CD3
 .36 CD2 CD3 : T B Daudi
 가 IL-2 가 T (LP-like T
) CD3 CD2
 .37 CD28 co-ligation CD2 ligation
 LP T CD3 CD2 ligation tyrosine
 ligation inositol triphosphate (IP3)가 phosphorylation profile LP T ,
 CD2 ligation IP3 가 LP T 가
 가 ,19 CD2 ligation 72 kDa . LP-like T DR+
 tyrosine CD2 ligation DR- LP-like T
 .20 CD2 CD3
 LP-like T CD2 CD3
 ligation protein tyrosine phosphorylation profile . : LP-like T
 Western blot . LP-like T DR+ DR- LP-like T magnetic cell
 SEB CD3 sorting .

tyrosine phosphorylation Western blot
 HLA DR IL-2R
 CD2 CD3
 DR+ LP-like T 80%
 CD45RO , 2/3 CD69,
 CD71 CD30
 DR- LP-like T
 10-20%
 LP-like T Western blot
 CD3 ligation 72 kDa
 tyrosine CD2 ligation
 LP-like T DR+ DR- LP-like T
 Western blot , CD2 ligation
 p72 가 DR+ LP-like T
 DR- LP-like T
 LP-like T CD2 ligation
 tyrosine phosphorylation profile

: T , LP-like T ,
 , CD2, Tyrosine phosphorylation

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