行政院國家科學委員會專題研究計畫　成果報告

以胃癌動物模式研究局部和周邊淋巴結的 CD4+CD25+調節性 T 細胞免疫調控機轉

計畫類別：個別型計畫
計畫編號：NSC94-2320-B-041-005-
執行期間：94 年 08 月 01 日至 95 年 07 月 31 日
執行單位：嘉南藥理科技大學嬰幼兒保育系

計畫主持人：陳怡伶
共同主持人：賴明德，沈延盛
計畫參與人員：方榮華

報告類型：精簡報告

處理方式：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中　華　民　國 95 年 10 月 11 日

Characteristics of CD4+CD25+ regulatory T cells in regional lymph nodes of mice with benzo[a]pyrene-induced forestomach carcinoma

Yi-Ling Chen[1], Jung-Hua Fang [2], Ming-Derg Lai[3], Yan-Shen Shan[2].

[1]Department of Childhood Education and Nursery, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan

[2]Department of Surgery, National Cheng Kung University Hospital,

[3]Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Correspondence: Yan-Shen Shan, MD PhD

Assistant Professor

Department of Surgery, National Cheng Kung University Hospital,

College of Medicine, National Cheng Kung University, Tainan, Taiwan.

138, Sheng-Li Road, Tainan 70428, Taiwan (ROC)

Tel: 886-6-2353535, ext 5181

Fax: 886-6-2766676

e-mail: ysshan@mail.ncku.edu.tw

Abbreviations used in this paper: Treg, regulatory T; B[a]P, benzo[a]pyrene; TILs, tumour-infiltrating lymphocytes.

Abstract

The increased populations of regulatory T cells in peripheral blood of gastric carcinoma reflect compromised host immunity. In this study, the murine model of benzo[a]pyrene induced forestomach carcinoma was used to analyze the distribution of regulatory T cells in different lymphoid tissues, including the regional lymph nodes, the peripheral lymph nodes, spleen, and thymus. The proportions of regulatory T cells in total $CD4^+$ T cells is clearly more increased in regional lymph nodes than other lymphoid tissues in benzo[a]pyrene-treated mice. These regulatory T cells expressed the Foxp3 transcript and protein and were dramatically enhanced in regional lymph nodes relative to peripheral lymph nodes. They were also enriched in $CD62L^-$ cell subset and expressed both CCR1 and CCR5 chemokine receptors. These results demonstrate that the accumulation of Treg cells in the regional lymph nodes mediated local suppressive immunity during progression of forestomach tumours.

Results

The inducible Treg cells of RLNs suppress local immunity via secretion of IL-10

Foxp3 is a crucial transcription factor and the specific marker for inducible regulatory T cells. Enumeration of isolated $CD4^+CD25^+$ (combined $CD25^{int}$ and $CD25^{high}$-expressing cells) and $CD4^+CD25^-$ T cells within RLNs, PLNs or thymus was shown in Figure 1-A. The expression of Foxp3 in Treg cells and $CD4^+CD25^-$ cells in the RLNs and PLNs of the B[a]P and control groups at various time points was examined. As expected, Treg cells of the RLNs and PLNs expressed high levels of Foxp3, while $CD4^+CD25^-$ T cells of the RLNs and PLNs expressed only low levels in the B[a]P group (Figure 1-B, -C, -D). Furthermore, the Foxp3 transcripts of Treg cells were dramatically enhanced in RLNs relative to PLNs at 32 weeks (Figure 1-D).

We further analyzed whether the $CD4^+CD25^+$ or $CD4^+CD25^-$ T cells of the RLNs and PLNs produce immunosuppressive cytokines such as IL-10 and TGF-β1. The two types of T cells of the RLNs and PLNs in the B[a]P-treated mice enhanced expression of IL-10 at transcription levels at 32 weeks (Figure 1-B, -C), but in control mice, just the $CD4^+CD25^+$ T cells of the RLNs expressed low level IL-10 transcripts at 32 weeks. The $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells of the RLNs and PLNs in B[a]P-treated and control mice produced little or no IL10 transcripts at 7 and 16 weeks (data not shown). We also examined the expression patterns of TGF-β1 in the $CD4^+CD25^+$ T cells and $CD4^+CD25^-$ T cells of the RLNs and PLNs in the B[a]P-treated and control mice. The expression of the TGF-β1 transcripts was negative at 7, 16 and 32 weeks, only the $CD4^+CD25^-$ T cells from the PLNs in B[a]P-treated mice produced low levels of the TGF-β1 transcripts at 32 weeks (Figure 1-B, -C).

To confirm the difference in proportion of Treg cells between RLN and PLN, we analyzed expression of Foxp3 by flow cytometric analysis (Figure 2-A, -B). The expression of Foxp3 in lymphocytes in RLNs was significantly higher than that in PLNs of B[a]P-treated mice at 22 weeks (Figure 2-C). Taking together, the Foxp3 expression in the $CD4^+CD25^+$ T cells suggests the activation of inducible Treg cells is associated with tumour formation. Moreover, Treg cells secrete IL-10, not TGF-β1, as a mediator for local inhibition of host immune functions during tumour progression.

Loss Expression of CD62L protein on Treg cells of RLNs

The down regulation of L-selectin (CD62L) expression from the cell surface depends upon cell activation. Loss of CD62L expression is a useful indicator for T cell maturation, activation or terminal differentiation. The percentage of $CD4^+CD62L^-$ cells in RLNs was higher than that in PLNs of B[a]P-treated mice at 22 weeks (Figure

3-B) and the percentage of $CD4^+$ $CD62L^+$ cells in PLNs, belong to the naïve pool, was higher than that in RLNs (Figure 3-C). Further, the percentage of $CD25^+CD62L^-$ cells in RLNs was higher than that in PLNs and no difference in $CD25^+CD62L^+$ between RLNs and PLNs (Figure 3-D, -E). The results implied that more Treg cells in the RLNs were at a different stage of maturation or activation.

Expression of CCR1 and CCR5 transcripts on Treg cells of RLNs

Chemokine receptors are important for T cell migration to lymph nodes, the expression of these receptors might distinguish characteristics on $CD4^+$ cell subsets of RLNs and PLNs. To distinguish which factor attract Treg cells migrate to RLNs, the expression of CCR1, CCR4, CCR5, CCR6, CCR7 and CCR8 transcripts on purified $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells of RLNs and PLNs were analyzed. According to the results of RT-PCR, $CD4^+CD25^+$ T cells of RLNs specifically expressed CCR1 and CCR5 mRNA of B[a]P-treated mice at 32 weeks (Figure 4). $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells of RLNs and PLNs of B[a]P-treated mice also exhibited highly expression of CCR4, CCR6, CCR7 and CCR8 mRNA. Those results suggested that Treg of RLNs in forestomach tumours might posses a unique chemotactic profile for Treg cells migrating to inflammation site which responses for local immune suppression during tumor progression.

Increased population of Treg cells in N1 RLNs of human gastric cancers

To confirm the similar local immune surveillance via the increased prevalence of Treg cells in N1 RLNs of gastric cancer, the Treg cells and total $CD4^+$ cells of PBMC, tumour tissues, and N1 regional lymph nodes from patients with early and advanced gastric cancer were examined. The frequency of Treg cells in total $CD4^+$ T cells in PBMC, tumour, and N1 RLNs was significantly higher in gastric cancer patients than

that in healthy donors, those in the tumour tissues and N1 RLNs were also significantly increased in advanced gastric cancer than that in early gastric cancer (Table 1). These findings confirm that the increase Treg cells in N1 RLNs is response for local immune dysfunction during progression of gastric cancer.

Figure Legends

Figure 1 $CD4^+CD25^+$ T cells express high level of Foxp3 transcripts. In **(A),** RLNs and PLNs $CD4^+CD25^-$ (P2) and $CD4^+CD25^+$ (P3) T cells were sorted and stained with FITC-anti-CD25 and PE-anti-CD4 mAbs. Foxp3, IL-10, TGF-β1 and β-actin mRNA expression of $CD4^+CD25^+$ and $CD4^+CD25^-$ cells in RLNs **(B)** and PLNs **(C)** in B[a]P and control mice at 32 weeks using RT-PCR. Lane 1 and 2: B[a]P mice. Lane 3 and 4: control mice. Lane 1 and 3: $CD4^+CD25^-$ cells. Lane 2 and 4: $CD4^+CD25^+$ cells. In (**D**), relative Foxp3 expression from $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells using real-time quantitative PCR is shown after normalization to HPRT expression.

Figure 2 The expression of Foxp3 protein of lymphocytes of RLNs (**A**) and PLNs (**B**) in B[a]P mice at 22 weeks. Foxp3 expression was determined as described in Materials and Methods. The histogram demonstrates FITC-conjugated Foxp3 staining (solid peak) and isotype control (hollow peak). In (**C**), the Foxp3 expression of RLNs vs PLNs were quantitated by flow cytometry. *P<0.05.

Figure 3 Cell-surface expression of CD62L on $CD4^+$ or $CD25^+$ cells between RLNs and PLNs in B[a]P mice at 22 weeks. CD62L expression was determined as described in Materials and Methods and quantitated by flow cytometry. The lymphocytes of RLNs vs PLNs were quantitated by flow cytometry in $CD4^+CD25^+$ (**A**), $CD4^+CD62L^-$ (**B**), $CD4^+CD62L^+$ (**C**), $CD25^+CD62L^-$(**D**), and $CD25^+CD62L^+$ (**E**). *P<0.05;

**P<0.01.

Figure 4 PCR analysis of chemokine receptor transcripts in $CD4^+CD25^-$ and $CD4^+CD25^+$ cells. CCR1, CCR4, CCR5, CCR6, CCR7, CCR8 and β-actin mRNA expression of $CD4^+CD25^-$ cells **(**lane 1, 3, 5, 7**)** and $CD4^+CD25^+$ **(**lane 2, 4, 6, 8**)** in RLNs **(**lane 1, 2, 3, 4**)** and PLNs **(**lane 5, 6, 7, 8**)** in B[a]P (lane 1, 2, 5, 6) and control (lane 3, 4, 7, 8**)** mice at 32 weeks using RT-PCR.

Table 1 The distribution of Treg cells in different tissues of gastric carcinoma patients

Stage of gastric cancer	**$CD4^+CD25^+$ (% of $CD4^+$ cells)**
Gastric cancer	
Early cancer (n=21)	
PBMC	**15.6 ± 3.4**
N1 regional lymph nodes	**11.1 ± 2.5**
Tumor tissues	**13.2 ± 4.0**
Advanced cancer (n=79)	
PBMC	**25.2 ± 3.0***
N1 regional lymph nodes	**26.5 ± 2.6***
Tumor tissues	**33.2 ± 4.7***
Healthy donor (n=7)	**8.3 ± 1.7**

*: P<0.05 compared to early cancer.

Figure 1

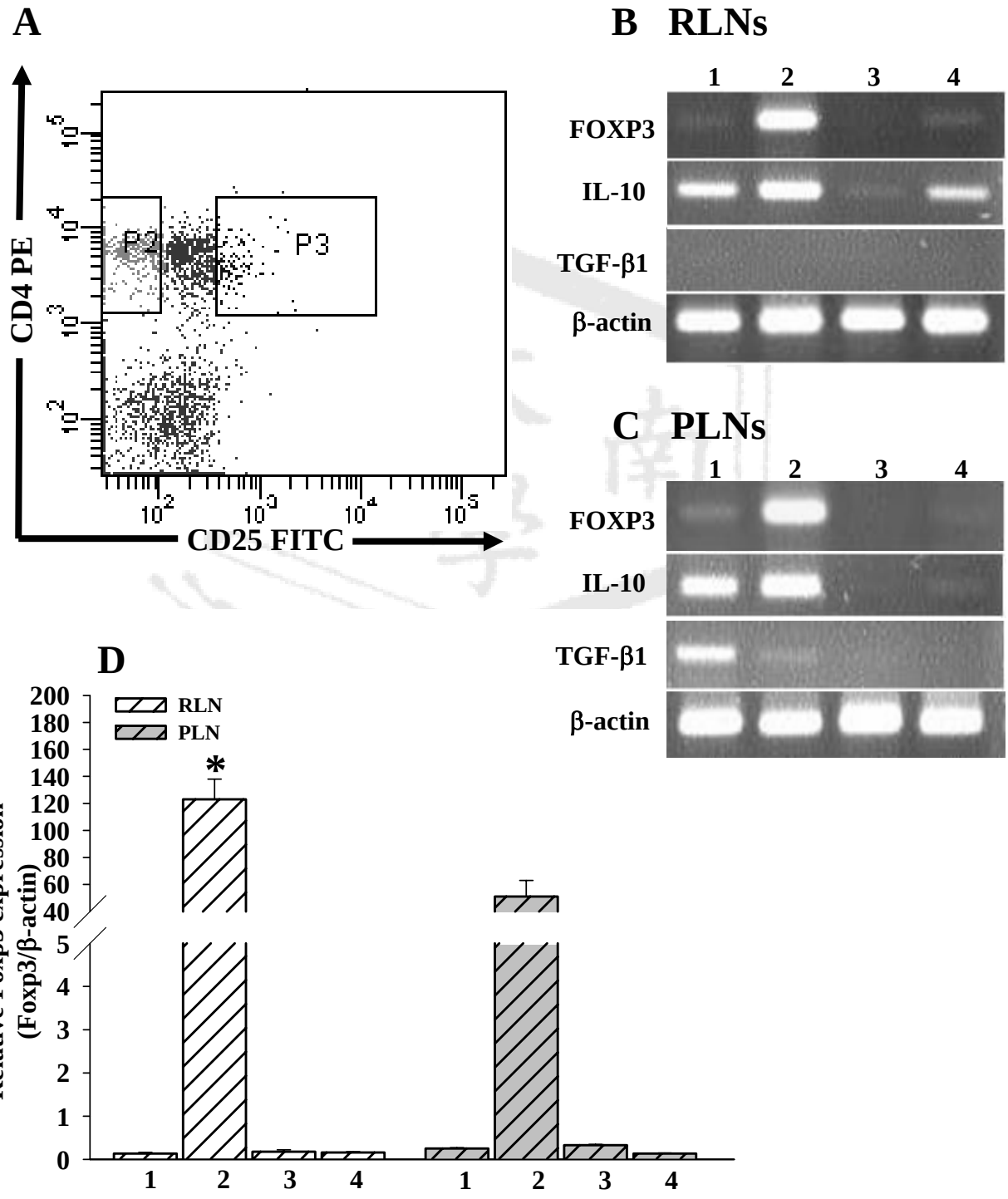
A
CD4 PE
CD25 FITC
P2
P3
B RLNs
1 2 3 4
FOXP3
IL-10
TGF-β1
β-actin
C PLNs
1 2 3 4
FOXP3
IL-10
TGF-β1
β-actin
D
RLN
PLN
*
Relative Foxp3 expression
(Foxp3/β-actin)
200
180
160
140
120
100
80
60
40
5
4
3
2
1
0
1 2 3 4 1 2 3 4

Figure 2

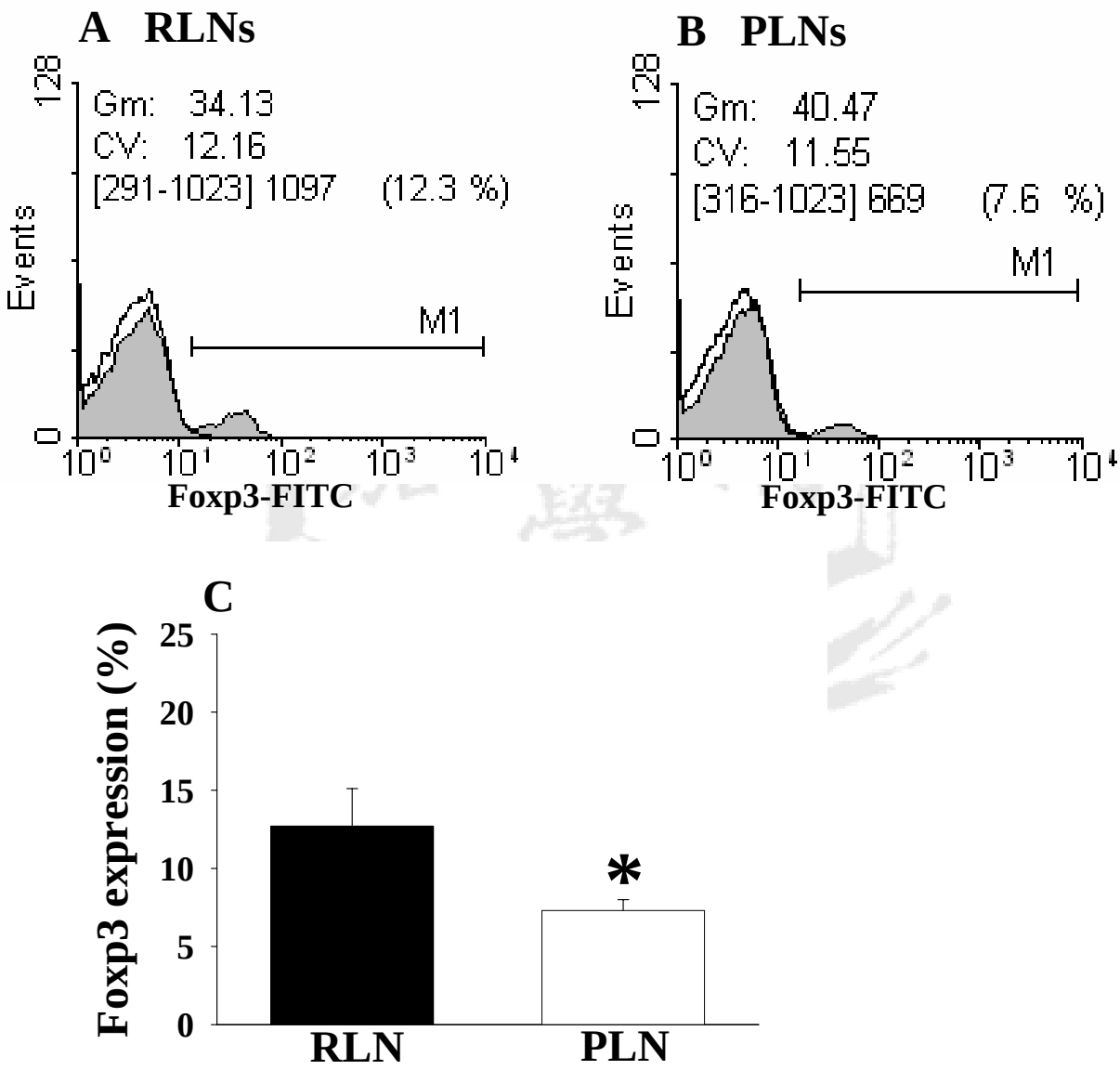
A RLNs
Gm: 34.13
CV: 12.16
[291-1023] 1097 (12.3 %)
M1
Events
128
0
Foxp3-FITC
B PLNs
Gm: 40.47
CV: 11.55
[316-1023] 669 (7.6 %)
M1
Events
128
0
Foxp3-FITC
C
Foxp3 expression (%)
25
20
15
10
5
0
*
RLN
PLN

Figure 3

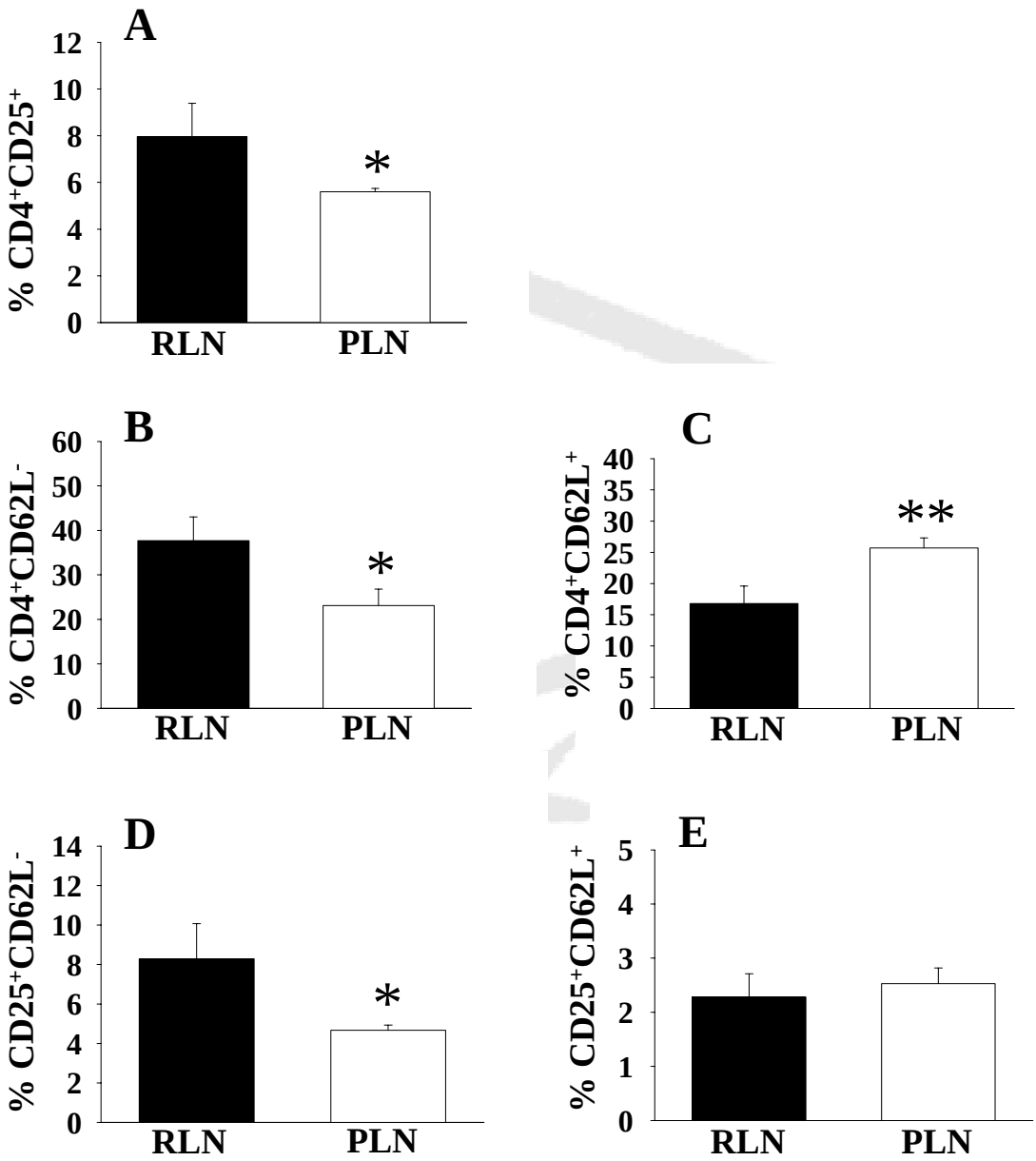
A
% CD4+CD25+
12
10
8
6
4
2
0
*
RLN
PLN
B
% CD4+CD62L-
60
50
40
30
20
10
0
*
RLN
PLN
C
% CD4+CD62L+
40
35
30
25
20
15
10
5
0
**
RLN
PLN
D
% CD25+CD62L-
14
12
10
8
6
4
2
0
*
RLN
PLN
E
% CD25+CD62L+
5
4
3
2
1
0
RLN
PLN

Figure 4