INCREASED LEVELS OF PERIPHERAL IGD^{-}CD27^{-} B CELLS IN PATIENTS WITH LUPUS NEPHRITIS CORRELATE WITH EARLY DIFFERENTIATED T LYMPHOCYTE SUBSETS

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INTRODUCTION

B Lymphocytes
CD19 IgD+ CD27-
CD19 IgD+ CD27+
CD19 IgD- CD27+
CD19 IgD- CD27-

Table 1.
Discrete double-negative (DN) B cell subsets.

Nomenclature	Phenotype	Prominence in (condition)	Properties	References
DN1	$CD19^+IgD^-CD27^-CD21^+$ $CD11c^-$ $T\text{-}bet^-\ CXCR5^+$	Elderly healthy individuals (aging)	Memory precursors	[16,17]
DN2	$CD19^+IgD^-CD27^-CD21^-$ $CD11c^+$ $T\text{-}bet^+CXCR5^-$	SLE (autoimmunity)	Extrafollicular ASC precursors	[17]
DN3	$CD19^+IgD^-CD27^-CD21^-$ $CD11c^-$ $[T\text{-}bet^{low}]$	COVID-19	??	[7,8]
atMEM/tbMEM	$CD19^+IgD^-CD27^-$ $CD21^-FcRL4^+$	Chronic infections	Exhausted, mucosal resident B cells	[35,36]

Sachinidis A. Mediterr J Rheumatol. 2021 Sep 30;32(3):192-199.

INTRODUCTION

Table 1
Human peripheral blood DN B cells in health and disease.

Condition	Phenotype	DN B cell subset	Technology	Alteration	Proposed functional role	Clinical correlations
Health	$CD19^{+}IgD^{-}CD27^{-}IgG^{+}$	DN1 proportion	Flow cytometry Ig V_H sequencing	/	Memory B cells	/
	$CD19^{+}IgD^{-}CD27^{-}$	Total	Ig V_H sequencing	/	SM B cells	/
	$CD19^{+}IgD^{-}CD27^{-}$	DN1-3	Single-cell RNA sequencing	/	DN1: SM precursors DN2/3: precursor of extrafollicular ASCs	/
Aging	$CD19^{+}IgD^{-}CD27^{-}$	Total	Flow cytometry Ig V_H sequencing	Increased in PB	Senescent memory B cells	/
Autoimmune diseases						
SLE	$CD19^{+}IgD^{-}CD27^{-}$	Total	Flow cytometry	Increased in PB	Memory B cells	Occurrence of nephritis, autoantibodies, disease activity
	$CD19^{+}IgD^{-}CD27^{-}CD11c^{-}CD21^{+}CXCR5^{+}$ $CD19^{bright}IgD^{-}CD27^{-}CD11c^{+}CD21^{-}CXCR5^{-}$	DN1 DN2	Flow cytometry RNA + Ig V_H sequencing	Increased DN2 in PB	DN1: SM precursors DN2: precursor of extrafollicular ASCs	Occurrence of nephritis, autoantibodies, disease activity
	$CD19^{+}IgD^{-}CD27^{-}CD11c^{-}CXCR5^{+}T\text{-}bet^{-}$ $CD19^{+}IgD^{-}CD27^{-}CD11c^{+}CXCR5^{-}T\text{-}bet^{+}$	DN1 DN2	Flow cytometry RNA sequencing	Increased DN2 in PB	Precursor of ASCs	Autoantibodies
	$CD19^{low}\ IgD^{-}CD27^{-}CXCR5^{-}$ $CD19^{int}\ IgD^{-}CD27^{-}CXCR5^{+}$ $CD19^{hi}\ IgD^{-}CD27^{-}CXCR5^{-}$	DN^{low} DN^{int} ~ DN1 DN^{hi} ~	Flow cytometry RNA sequencing	Increased DN^{low}, DN^{int} and DN^{high} in PB	DN^{low} + DN^{hi}: Precursor of ASCs DN^{int}: memory B cells	ND

Beckers, L. Immunol. Lett. ***2023****, 255, 67–76.*

INTRODUCTION

Correlation with LN and activity

frontiers in Medicine

ORIGINAL RESEARCH
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Double Negative B Cell Is Associated With Renal Impairment in Systemic Lupus Erythematosus and Acts as a Marker for Nephritis Remission

Xujie You [1,2], Ruijun Zhang [1,2], Miao Shao [1,2], Jing He [1,2], Jiali Chen [1,2], Jiajia Liu [1,2], Xia Zhang [1,2], Xu Liu [1,2], Rulin Jia [1,2], Xiaolin Sun [1,2] and Zhanguo Li [1,2]*

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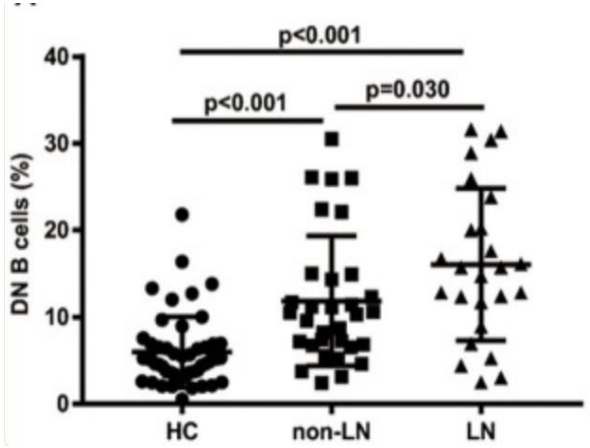

You X et al, Front Med (Lausanne). 2020 Apr 7;7:85

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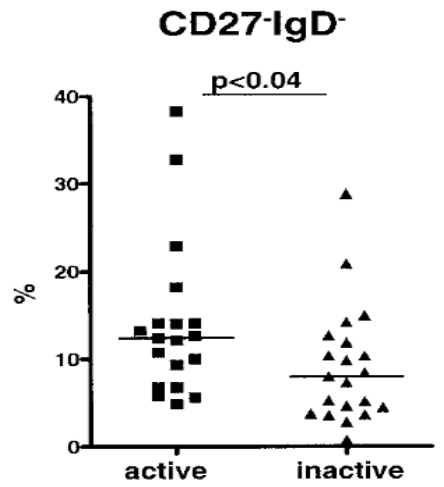
Vol. 58, No. 6, June 2008, pp 1762–1773
DOI 10.1002/art.23498

Activated Memory B Cell Subsets Correlate With Disease Activity in Systemic Lupus Erythematosus

Delineation by Expression of CD27, IgD, and CD95

Annett M. Jacobi,[1] Karin Reiter,[2] Meggan Mackay,[3] Cynthia Aranow,[3] Falk Hiepe,[4] Andreas Radbruch,[5] Arne Hansen,[2] Gerd-R. Burmester,[2] Betty Diamond,[3] Peter E. Lipsky,[6] and Thomas Dörner[4]



Jacobi AM et al, Arthritis Rheum. 2008 Jun;58(6):1762-73

AIM

Aim of the present study was to evaluate the possible correlation of DN B lymphocytes with T lymphocyte alterations, in SLE patients.

MATERIAL AND METHODS (1)

Cross-sectional study

30 SLE patients and 31 Healthy Controls (HC) of similar age, sex, and ethnicity

Inclusion criteria:

- Adults aged 18–67 years
- SLE diagnosis based on the SLICC/ACR 2012 criteria
- In remission, with no flare-ups for at least 2 years

Exclusion criteria:

- Diabetes mellitus, malignancy, hematological disorder, impaired renal function (eGFR < 60 mL/min/m2)
- Presence of chronic, active or recent (<6 months) infection
- Therapy with monoclonal antibodies in the past or cyclophosphamide during the last 24 months

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MATERIAL AND METHODS (2)

- The expression of IgD and CD27 on B lymphocytes and of CD45RA, CCR7, CD28, CD31, and CD57 on T lymphocytes, on both CD4 and CD8, was assessed using flow cytometry. The expression of the above surface molecules examined determined divergent subpopulations, distinct naïve and senescent B and T lymphocyte subtypes.

B Lymphocytes
CD19 IgD+ CD27-
CD19 IgD+ CD27+
CD19 IgD- CD27+
CD19 IgD- CD27-

T Lymphocytes (CD4+/CD8+)	
Naïve and Early differentiated cells	CD45RA+ CCR7+ CD31+ CD28+ CD57-
Central Memory	CD45RA- CCR7+
Effector Memory	CD45RA- CCR7-
Senescent/Advanced differentiated cells	CD45RA+ CCR7- CD28-CD57- CD28- CD57+

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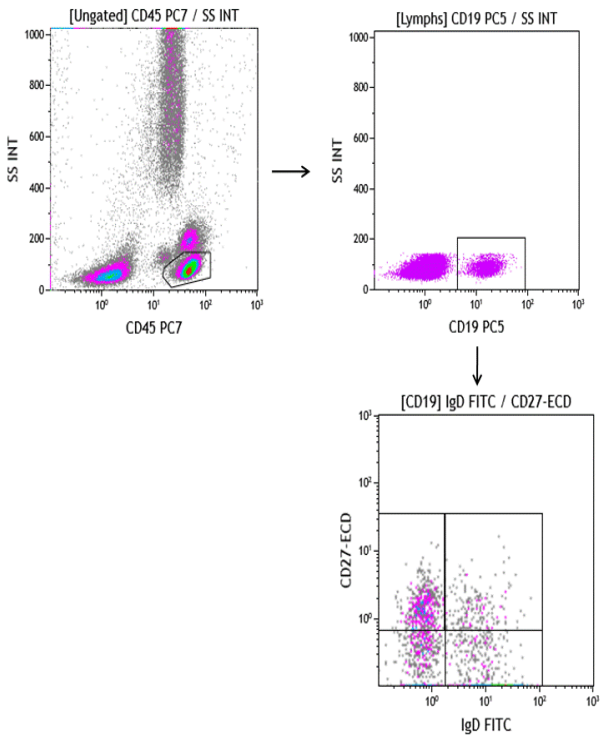
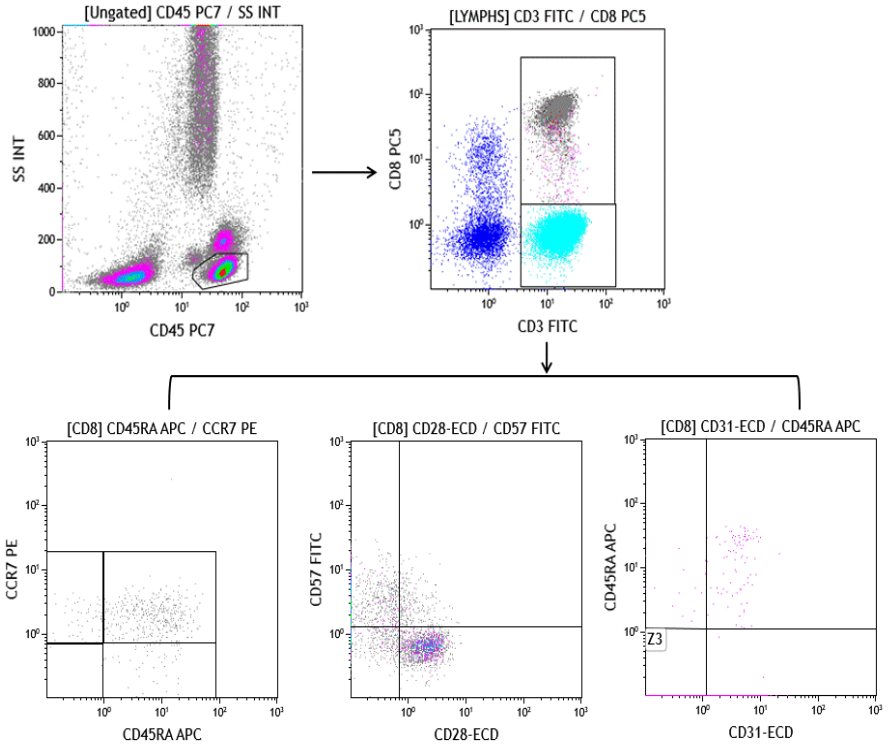
MATERIAL AND METHODS (3)

Gating strategy

B lymphocytes



T lymphocytes



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CHARACTERISTICS

	SLE	HC	*p*
n	30	31	
Age (years)	43±14	49±13	NS
WBC (cell/μL)	7200±3350	6400±1800	NS
Neutrophils (cell/μL)	4600±3500	3500±1200	0.03
Lymphocytes (cell/μL)	1400±900	2100±900	0.005
Serum urea (mg/dl)	36±12	34±5	NS
Serum creatinine (mg/dl)	0.9±0.5	0.87±0.6	NS
Time since diagnosis (months)	84 (45-125)	-	-
Lupus Nephritis (n)	23	-	-
C3 (mg/dl)	74.65 (29.9-127)	-	-
C4 (mg/dl)	15.7 (3.86-27.2)	-	-
SLEDAI score (at diagnosis)	10 (2-18)	-	-
SLEDAI score (on evaluation)	2 (1-5)	-	-
Uprot (mg/24h)	680 ± 5.600	-	-

SLE			
Therapy	n	%	Time
Hydroxychloroquine	30	100	84 (45-125)
Prednisolone	24	80	78 (45-92)
MMF	18	60	53 (37-65)
CNIs	9	30	42 (28-56)
MMF+CNIs	5	16.7	

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	SLE	HC	p
n	30	31	
CD19 (%)	7.9 (2.1–28.6)	11.8 (5.4–24)	0.012
CD19 cells/μL	75.4 (14.4–520.8)	214 (84–576)	<0.001
IgD+CD27- (%)	51.5 (0.4–94)	58.7 (4.5–86.9)	0.34
IgD+CD27- cells/μL	37.71 (0.26–434.84)	117 (5–364)	<0.001
IgD+CD27+ (%)	3.9 (0.2–22)	8.4 (1.5–44)	0.014
IgD+CD27+ cells/μL	5.12 (0.13–17.55)	23 (2–700)	<0.001
IgD-CD27+ (%)	19.1 (2.2–78)	17.9 (7.1–71.9)	0.7
IgD-CD27+ cells/μL	18.58 (0.47–89.58)	38 (11–258)	0.001
IgD-CD27- (%)	12.9 (2.3–74.2)	8 (1.7–35)	0.04
IgD-CD27- cells/μL	10.84 (0.93–122.91)	21 (3–202)	0.007
Ratio DN/[(IgD+CD27-) + (IgD-CD27+) + (IgD+CD27+)]	0.14 (0.02–2.9)	0.08 (0.02–0.54)	$p = 0.04$

	SLE	HC	p
n	30	31	
CD4 (cells/μL)	651.2 (71.1–1478.2)	986 (344–1591)	0.004
	Early differentiated cells		
CD4+CD31+	216.38 (16.3–904.7)	250 (69–967)	0.14
CD4CD45RA+CD28+	267.97 (20.62–1030.31)	388 (139–1402)	0.02
CD4CD45RA+CD57-	254.03 (21.05–1077.61)	401 (160–1373)	0.035
CD4CD45RA-CD57-	290.67 (38.96–884.43)	539 (173–991)	<0.001
CD4CD28+CD57-	610.7 (54.68–1461.94)	958 (332–1569)	0.004
CD4CD28+CD57+	4.7 (0–806)	7 (0–245)	0.21
	Memory cells		
CD4CD45RA-CCR7+	402.35 (38.7–972.4)	563 (40–1001)	0.046
CD4CD45RA-CCR7-	1.62 (0–73.49)	11 (0–590)	0.002
	Advanced differentiated/senescent cells		
CD45RA+CCR7-	7.29 (0–180.62)	23 (0–487)	0.027
CD4CD28-	20.12 (1.27–139.06)	38 (3–299)	0.04
CD4CD28-CD57+	9.90 (0.46–73.8)	23 (0–274)	0.1
CD45RA+CCR7-CD28-	1.2 (0–82)	2.5 (0–106)	0.21

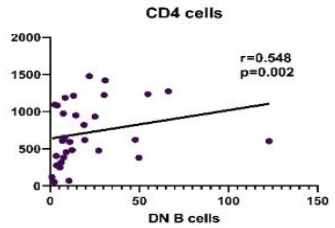
RESULTS

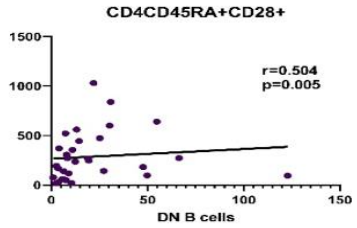
- Phenotypic Analysis of B and T Lymphocytes in Patients with SLE and in HC

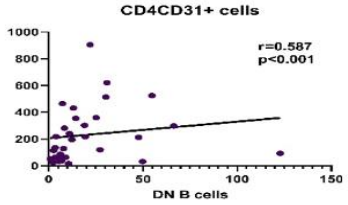
	SLE	HC	p
n	30	31	
CD8 (cells/μL)	414.8 (60.6–2017.8)	454.5 (154–1310)	0.26
	Early differentiated cells		
CD8+CD31+	88.19 (8.2–1047)	187.5 (8–541)	0.26
CD8CD45RA+CD28+	113.56 (1.81–753.7)	212.5 (7–1257)	0.17
CD8CD45RA+CD57-	63.65 (3.83–889.8)	133 (8–552)	0.17
CD8CD45RA-CD57-	194.52 (1.8–945.1)	179 (28–555)	0.99
CD8CD28+CD57-	249.45 (5.49–1362)	298 (95–646)	0.1
CD8CD28+CD57+	12(0.4–132)	8.5 (0–424)	0.58
	Memory cells		
CD8CD45RA-CCR7+	171.52 (2.5–1417)	123 (1–941)	0.14
CD8CD45RA-CCR7-	13.94 (0.59–92.37)	25 (0–355)	0.53
	Advanced differentiated/senescent cells		
CD8CD45RA+CCR7-	11.13 (0–279.6)	49.5 (0–534)	0.02
CD8CD28-	87.83 (4.56–1361.2)	135 (36–633)	0.14
CD8CD28-CD57+	53.17 (0.83–571.04)	71 (0–470)	0.17
CD45RA+CCR7-CD28-	37.3 (2.1–263)	197 (9–783)	<0.0001

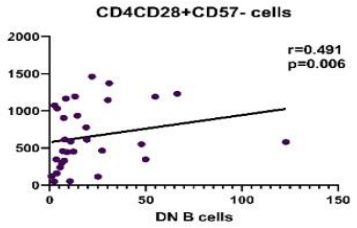
RESULTS

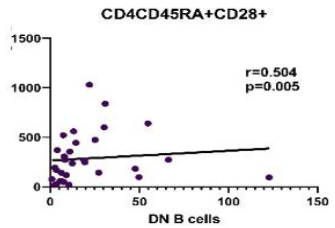
CD4 T lymphocytes

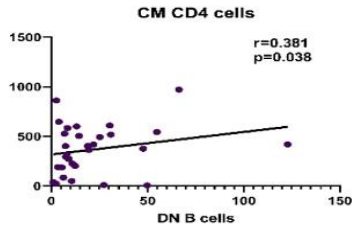




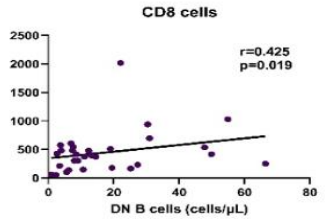


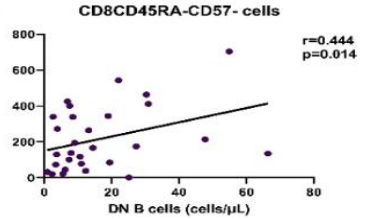


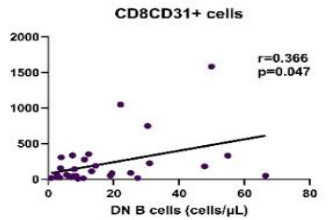


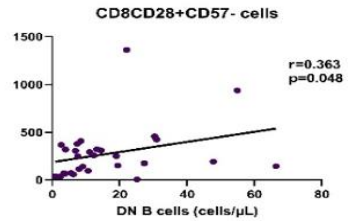


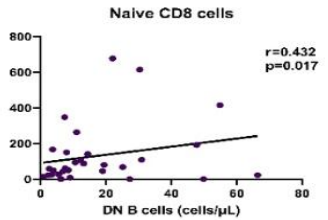
CD8 T lymphocytes

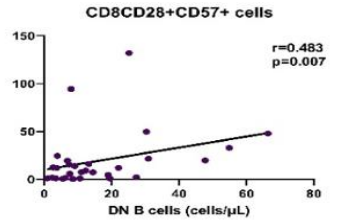












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CONCLUSIONS

- Double negative B lymphocytes predominate in the peripheral blood of SLE patients, even at remission
- Their population is closely associated with early differentiated T lymphocyte subsets, indicating a potential causality role of DN B cells in T lymphocyte activation
- We point the need to further assess changes in lymphocyte subpopulations and their interactions during disease evolution and regression following response to treatment

THANK YOU !