Activation of cytotoxic T-cell receptor γδ T lymphocytes in response to specific stimulation in myelodysplastic syndromes

Jean-Jacques Kiladjian,[1,2] Géraldine Visentin,[1] Emilie Viey,[1] Sylvie Chevret,[1] Virginie Eclache,[4] Jerome Stirnemann,[5] Jean Henri Bourhis,[1,6] Salem Chouaib,[3] Pierre Fenaux,[2] and Anne Caignard[1]

[1]INSERM U753, Institut Gustave Roussy, Villejuif; [2]AP-HP, Hôpital Avicenne, Service d'Hématologie Clinique, Bobigny, and Université Paris 13; [3]APHP, Hôpital Saint-Louis, DBIM, Paris; [4]APHP, Hôpital Avicenne, Laboratoire d'Hématologie, Bobigny; [5]APHP, Hôpital Jean Verdier, Service de Medecine Interne, Bondy; [6]Institut Gustave Roussy, Service d'Hématologie, Villejuif, France

Supplementary Table 1.

Table 1. Clinical and hematologic characteristics of the MDS patients analyzed

Patient	*% γδ2 in PBMC at Day 0*	*Response to BrHPP*	*WHO*	*WBC (G/l)*	*% BM blasts*	*Karyotype*	*IPSS*	*Risk category*	*AID*	*Follow-up (months)*
MDS1	0.2	0	RAEB1	1.9	2	4	1	INT-1	0	5
MDS2	0.21	0	RAEB1	7	8	2	1	INT-1	0	6
MDS3	2.9	1	RAEB1	2.3	8	1	0.5	INT-1	0	15
MDS4	2.82	1	5q- Sd	3.9	8	4	0	Low	0	9
MDS5	1.23	1	RAEB2	1.6	13	2	1	INT-1	0	30
MDS6	10.5	1	RAEB2	2.7	11	1	2	INT-2	0	21
MDS7	3.85	1	RCMD	3.8	3	3	1	INT-1	1	11
MDS8	9.96	1	RCMD	NA	NA	NA	NA	NA	NA	NA
MDS9	0.43	0	RAEB2	4.4	14	4	2.5	High	1	3
MDS10	0.48	0	RCMD	8.3	4	4	0	Low	1	73
MDS11	0.11	0	RCMD	4.4	2	1	0	Low	0	12
MDS12	7.51	1	RAEB2	1.2	15	1	2	INT-2	0	14
MDS13	0.27	0	5q- Sd	3.7	2	4	0.5	INT-1	0	3
MDS14	3.03	1	MDS-U	4	NA	NA	NA	NA	0	13
MDS15	0.1	1	RAEB2	2.1	19	1	1.5	INT-2	0	0
MDS16	4.5	1	5q- Sd	2.9	2	4	0.5	INT-1	0	38
MDS17	0.19	1	RAEB1	4.1	6	4	1	INT-1	0	5
MDS18	0.39	0	RAEB2	25.86	17	2	1.5	INT-2	0	11
MDS19	1.94	1	RAEB2	NA	NA	NA	NA	NA	NA	NA
MDS20	2.5	NA	RA/RARS	2	4	2	1	INT-1	0	132
MDS21	0.001	1	RCMD	3.5	4	1	0.5	INT-1	1	53
MDS22	2.2	1	RAEB1	3.5	8	4	1	INT-1	0	14
MDS23	1.16	1	RA/RARS	3.9	3	2	0.5	INT-1	0	21
MDS24	9.47	0	RA/RARS	NA	NA	NA	NA	NA	NA	NA
MDS25	NA	0	RCMD	3	1	NA	NA	NA	1	72
MDS26	0.2	1	MDS-U	1.7	3	NA	NA	NA	1	55
MDS27	1.15	0	5q- Sd	3.1	2	4	0.5	INT-1	0	79
MDS28	0.1	1	MDS/MPD	17.4	4	1	0	Low	0	54
MDS29	0.1	1	RAEB1	2.6	11	3	2	INT-2	1	72
MDS30	0.9	1	RAEB1	2.3	8	3	2	INT-2	0	169
MDS31	1.29	1	RAEB1	3.6	5	2	1.5	INT-2	0	11
MDS32	2.61	0	RAEB2	3.9	11	3	2.5	High	1	25
MDS33	0.41	0	RCMD	3.4	3	1	0	Low	0	9
MDS34	0.06	0	RAEB1	1.4	7	1	1	INT-1	1	28
MDS35	0.08	0	RAEB2	3.7	15	3	2.5	High	0	14
MDS36	4.7	1	RAEB1	7	9	2	0.5	INT-1	0	3
MDS37	0.2	1	RA/RARS	5.6	1	2	0.5	INT-1	0	9
MDS38	1.44	0	RAEB1	NA	NA	NA	NA	NA	NA	NA
MDS39	0.02	0	RCMD	8.3	3	4	0	Low	1	65
MDS40	2.1	1	RA/RARS	8.2	1	2	0.5	INT-1	0	13
MDS41	0.1	0	RAEB1	5	NA	4	NA	NA	0	13
MDS42	8.72	1	RCMD	3.7	3	3	1.5	INT-2	0	14
MDS43	2.54	1	RAEB1	12.6	8	1	0.5	INT-1	0	13
MDS44	12.69	1	RAEB2	1.9	14	3	2.5	High	0	22

%Vδ2 indicates the percentage of circulating Vδ2T cells before stimulation with BrHPP (assessed using the CD3/TCRV‰2 combination). PBMC were stimulated with BrHPP (3 μM) and IL-2 (100 U/mL) for 8-12 days. During culture, γδ T-cell expansion was determined by assessing the percentage of CD3Vδ2 T cells by flow cytometry every 3 days. Response to BrHPP : 0 indicates <30% expansion and 1 indicates 30-98% expansion. NA: not available. WHO: World Health Organization classification. RA: refractory anemia. RARS: RA with ringed sideroblasts. RAEB: RA with excess of blasts. RCMD: refractory cytopenia with multilineage dysplasia. MDS-U: myelodysplastic syndrome-unclassifiable. 5q- Sd: 5q- syndrome. MPD: myeloproliferative disorder. WBC: white blood cells. %BM blasts: percentage of blasts in the bone marrow. IPSS: International Prognostic Scoring System. INT-1 and -2: intermediate-1 and -2 risk categories according to the IPPS score. AID: autoimmune disease; 1 indicates patients presenting concomitant AID.

Supplementary Immunophenotypc study

Immunophenotypic study

PBMC ($2x10^5$) were incubated at 4°C for 30 min with a combination of the following fluorescein isothiocyanate- (FITC), phycoerythrin- (PE) and phycoerythrin-cyanin 5- (PC5) conjugated antibodies to determine the percentages of αβ and γδ T cells: CD3-PE (UCHT1, IgG1), Vδ2-FITC (Immu 389, IgG1), CD8-PC5 (B9.11, IgG1), CD45RA, CD45RO, and CD27 purchased from Immunotech (Marseille, France). The cells were then cultured as described above. Between days 8 and 14 of culture, the distribution of naïve and memory subsets of expanded Vγ9Vδ2 T cells were analyzed by triple staining using CD8-ECD (SFCI21 ThyD3, IgG1, Coulter), CD3-APCCy7 (SK7, IgG1, BD Pharmingen) CD45RA-APC (HI100, IgG2b, BD Pharmigen), and CD27-PE (1A4, IgG1, Immunotech) monoclonal antibodies. Background levels of staining were measured using isotypic controls. Compensation was set up with single-stained samples. Low forward scatter elements (red cells and debris) were excluded from the analysis and 10,000 events were collected and analyzed on a FACSsort machine (Becton Dickinson, Pont de Chaix, France) using Cell Quest software (Becton Dickinson). For repertoire studies, only samples with >1% of γδ T cells were analyzed (as below this value, very low numbers of events in each subclass do not allow confidently reproducible results).

Citation: Kiladjian J-J, Visentin G, Viey E, Chevret S, Eclache V, Stirnemann J, Bourhis JH, Chouaib S, Fenaux P, and Caignard A. Activation of cytotoxic T-cell receptor γδ T lymphocytes in response to specific stimulation in myelodysplastic syndromes. Haematologica 2008 Mar; 93(3):381-389. doi: 10.3324/haematol.11812