Letters to the Editor

Oligoclonal expansion of T cells in persistent oligoarticular juvenile idiopathic arthritis

Sirs,

Juvenile idiopathic arthritis (JIA) summarises a group of clinical heterogeneous autoimmune diseases. (1) The different subtypes of JIA are characterised by different pathogenic T cell subtypes, characterised by different T cell receptors (TCR) (2). Studies of the TCR performed with monoclonal antibodies showed expression of Vβ5, Vβ14 and Vβ20 chains in synovial fluid (SF) of patients with JIA (2-4). The aim of this study was to demonstrate an oligoclonal expansion of T cells in the peripheral blood (PB) and/or SF in patients with persistent oligoarticular onset (oJIA) of JIA.

All patients were seen at two tertiary care centres. Diagnosis of JIA was based on the revised ILAR criteria (5). Matched pairs of samples were investigated derived from PB and SF of 9 patients (mean age 8.6 years) with active persistent oJIA. Samples from PB and SF from two patients with Lyme arthritis (LA) were analysed as a reference. The diagnosis of LA was made in cases of arthritis and positive antibodies to Borrelia burgdorferi detected by a ELISA and immunoblotting (6). The study was performed according to the declaration of Helsinki 2000 and approved by the local ethics committee, Friedrich-Alexander University Erlangen-Nuremberg. All patients and/or their parents had been given their written informed consent.

Leucocytes and C-reactive protein (CRP) were normal in all patients. Antinuclear antibodies were positive in eight patients. JIA patients were treated with non steroidal antiinflammatory drugs (NSAID) and 1 patient with methotrexate. Peripheral blood mononuclear cells (PBMC) from PB (5–10ml, heparinised blood) and intraarticular mononuclear cells (IAMC) from SF (10ml anticoagulated with heparine) were prepared according to standard procedures (Lymphoprep, Nycomed Pharma AS, Asker, Norway). The cells were studied by multicolour flow cytometry (IOTestBetaMark, Immunotech, 13276 Marseille, France). First $CD3^+$ positive, $CD4^+$ and $CD8^+$ positive cells and CD4CD8 negative (double negative (dn)) and CD4CD8 positive (double positive (dp)) cells were identified with monoclonal antibodies labelled with phycoerythrin-cyanin 5 (PC5) and phycoerythrin-Texas Red (ECD). Afterwards, the antibodies against TCR β-chain were investigated. Results were expressed as the percentage of gated lymphocytes. The *t*-test for independent variables was used to compare results in PB and SF (SPSS version 15.0; SPSS, Chicago, IL).

The CD4/CD8 ratio in the IAMC were found to have similar ranges (0,91-3,61) as in PBMC (1.45–3.27). Analyses on the distribution of certain Vß subgroups showed a statistically significant increased frequency of CD4 T cells bearing Vβ2, Vβ5.1, and Vβ17 in PBMC ($p<0.0001$). The IAMC fraction presented a significant predominance of Vβ2 and Vβ5.1 CD4 T cells ($p<0.0001$) (Fig. 1a). In CD8 cells Vβ 2, Vβ3, Vβ13.1 and Vβ13.2 were found extremely statistically significant increased within the PBMC ($p<0.0004$). Vβ1, Vβ2, Vβ12 and Vβ13.2 dominated extremely statistically significant the IAMC fraction in all patients ($p<0.0001$) (Fig. 1b). The most remarkable statistically very significant differences between IAMC and PBMC were observed in CD4/8 dn T cells with increased frequencies of Vβ3, Vβ8 and Vβ20 in IAMC ($p<0.0039$). These differences were not observed to correlate with age, sex, disease duration and treatment. Moreover, the patient receiving methotrexate did not show any remarkable distribution of T cell subtypes. The most pronounced differences comparing JIA and LA patients were the increased presentation of CD8 cells with Vβ5.3, Vβ12 and Vβ13.1 in IAMC of LA patients. These results were not statistically significant. All patients are in clinical remission (Table I).

This is the first study which inverstigated TCR subsets related to T cell subpopula-

Table I. Clonotypic archetypes in the T cell repertoire of persistent oligoarticular juvenile idiopathic arthritis (oJIA) and Lyme arthritis (LA). The vials (numbers 1-8) contain three conjugated TCR Vβ antibodies: the first is conjugated to fluoreszeinisothiocyanat (FITC), the second one to phycoerythrin (PE) and the third one is conjugated to both of them, FITC and PE. The results were expressed as the percentage of $CD4^+$ and $CD8^+$ positive, $C4^{++}$ and $CD8^{++}$ high positive cells and CD4CD8 negative (double negative (dn)) and CD4CD8 positive (double positive (dp)) cells of the gated CD3 positive lymphocytes in the peripheral blood (PB) and synovial fluid (SF). The accounts are the mean of all patients with persistent oligoarticular JIA and LA.Values in bold are the most remarkable results.

	Vial	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	5	6	6	6	7	7	7	8	8	8
	subset Vb	5.3	7.1	3	9	17	16	18	5.1	20	13.1	13.6	8	5.2	2	12	23	1	21.3	11	22	14	13.2	4	7.2	
	label	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE	FITC	FITC+PE	PE		
oJIA	$CD4^{++}$	0.34	0.44	0.92	0.85	**1.37**	0.21	0.37	**1.34**	0.62	0.88	0.65	0.71	0.54	**2.40**	0.57	0.19	0.82	0.37	0.38	1.03	0.47	0.73	0.37	0.32	
PB	$CD4^+$	0.23	0.38	1.28	0.33	**1.08**	0.44	0.14	**1.35**	0.91	0.72	0.41	0.42	0.18	**1.65**	0.80	0.06	0.72	0.42	0.18	0.54	0.33	0.61	0.11	0.22	
	$CD8^{++}$	0.12	0.49	**0.61**	0.17	0.56	0.34	0.04	0.30	0.28	**0.52**	0.28	0.23	0.17	**0.72**	0.34	0.17	0.74	0.38	0.11	0.32	0.42	**0.58**	0.19	0.24	
	$CD8^+$	0.08	0.28	**0.42**	0.09	0.27	0.35	0.02	0.35	0.47	**0.42**	0.17	0.13	0.10	**0.45**	0.52	0.05	0.66	0.39	0.15	0.34	0.46	**0.42**	0.04	0.15	
	dn	0.03	0.10	0.49	0.08	0.17	0.49	0.04	0.17	**0.63**	0.09	0.14	0.50	0.06	0.40	**0.58**	0.04	0.11	0.29	0.20	0.20	0.19	0.18	0.06	0.48	
	DP	0.06	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.01	**0.41**	0.01	0.00	0.09	0.03	0.01	0.03	0.05	0.00	0.05	0.01	0.01	0.10	0.01	0.01	
SF	$CD4^{++}$	0.59	0.44	0.77	1.01	1.13	0.32	0.37	**1.16**	0.63	0.90	0.50	0.58	0.79	**2.29**	0.65	0.34	0.97	0.52	1.03	1.06	0.55	0.82	0.38	0.32	
	$CD4^+$	0.21	0.36	0.99	0.26	0.71	0.55	0.12	**1.14**	0.88	0.63	0.49	0.69	0.20	**1.25**	0.89	0.09	0.82	0.56	0.10	0.37	0.29	0.48	0.14	0.41	
	$CD8^{++}$	0.14	0.38	0.42	0.18	0.38	0.32	0.04	0.31	0.29	0.37	0.22	0.21	0.25	**0.73**	**0.56**	0.15	**0.70**	0.36	0.21	0.31	0.51	**0.72**	0.12	0.21	
	$CD8^+$	0.07	0.34	0.45	0.04	0.19	0.30	0.03	0.21	0.33	0.34	0.25	0.19	0.09	**0.44**	**0.74**	0.06	**0.42**	0.38	0.09	0.35	0.40	**0.40**	0.05	0.19	
	dn	0.12	0.47	**2.07**	0.19	0.53	1.33	0.10	0.44	1.47	0.26	0.33	**2.20**	0.12	0.51	1.81	0.07	0.26	1.05	0.43	0.41	1.06	0.20	0.34	1.29	
	DP	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.04	0.01	**0.28**	0.03	0.01	0.09	0.06	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.07	0.03	0.02	
LA	$CD4^{++}$	0.42	1.15	0.33	0.83	1.63	0.51	0.21	2.26	0.71	1.01	0.61	1.36	0.66	3.42	1.36	0.13	1.06	1.01	0.40	1.48	0.42	0.99	0.67	0.33	
PB	$CD4^+$	0.11	0.25	0.19	0.58	0.90	0.19	0.11	0.87	0.33	0.35	0.33	0.68	0.14	0.85	0.30	0.08	0.57	0.25	0.12	0.49	0.20	0.37	0.24	0.15	
	$CD8^{++}$	0.11	0.66	0.68	0.20	0.73	0.56	0.04	0.32	0.30	0.60	0.18	0.47	0.18	0.40	0.52	0.18	0.52	0.42	0.11	0.39	0.16	0.38	0.20	0.24	
	$CD8^+$	0.11	0.23	0.05	0.19	0.61	0.15	0.04	0.24	0.18	0.94	0.18	0.47	0.15	0.33	0.19	0.21	0.59	0.38	0.14	0.32	0.29	0.38	0.15	0.15	
	dn	0.09	0.31	0.68	0.47	0.60	0.38	0.11	0.81	0.58	0.35	0.35	0.91	0.11	0.76	0.56	0.06	0.69	1.02	0.30	0.65	0.34	0.41	0.25	0.44	
	DP	0.08	0.01	0.00	0.01	0.03	0.01	0.02	0.02	0.00	0.45	0.01	0.01	0.18	0.02	0.00	0.03	0.02	0.01	0.03	0.02	0.01	0.07	0.01	0.01	
SF	$CD4^{++}$	0.56	0.81	0.95	1.12	1.15	0.82	0.51	1.99	1.47	1.75	0.67	1.01	0.89	2.23	2.57	0.30	1.14	1.12	0.76	1.33	0.52	1.26	0.52	0.54	
	$CD4^+$	0.15	0.38	0.44	0.53	0.38	0.23	0.12	0.83	0.69	0.44	0.26	0.62	0.12	0.82	0.53	0.06	0.32	0.52	0.13	0.35	0.25	0.27	0.13	0.41	
	$CD8^{++}$	**0.24**	0.75	0.41	0.62	0.79	0.79	0.13	0.82	0.55	**0.56**	0.27	0.28	0.21	0.55	**1.59**	0.16	0.59	0.64	0.14	0.68	0.49	0.82	0.14	0.43	
	$CD8^+$	**0.20**	0.32	0.09	0.13	0.38	0.59	0.04	0.39	0.20	**0.46**	0.22	0.19	0.13	0.44	**1.17**	0.18	0.53	0.46	0.08	0.43	0.48	0.30	0.05	0.15	
	dn	0.08	0.28	0.93	0.18	0.45	0.83	0.03	0.41	1.05	0.29	0.27	1.19	0.09	0.50	1.02	0.03	0.32	1.14	0.19	0.38	0.54	0.16	0.17	0.82	
	DP	0.02	0.01	0.01	0.02	0.01	0.00	0.00	0.02	0.01	0.17	0.01	0.00	0.07	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.08	0.02	0.0	

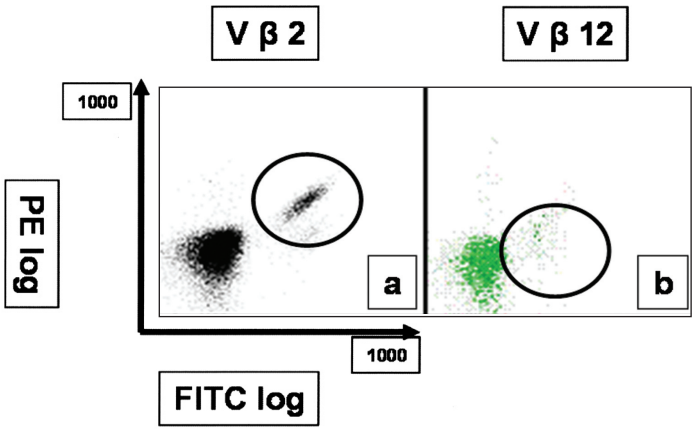

Fig. 1. Vβ2 positive CD4 T cells were increased within PBMC (**a**). In the IAMC Vβ12 (**b**) positive CD8 T cells were a dominating cell population in all patients.

tions in one JIA subgroup. However, as the T-cell repertoire is a highly polymorphic system, our results have to be proved on a larger cohort of patients and have to be investigated in patients with extended oJIA and polyarticular JIA.

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