



HAL
open science

The turnover of synovial T cells is higher than in T cells in the peripheral blood in persistent oligoarticular juvenile idiopathic arthritis

Juergen Brunner, Martin Herrmann, Markus Metzler, Udo Gaipl, Gert Reuter, Johannes-Peter Haas

► To cite this version:

Juergen Brunner, Martin Herrmann, Markus Metzler, Udo Gaipl, Gert Reuter, et al.. The turnover of synovial T cells is higher than in T cells in the peripheral blood in persistent oligoarticular juvenile idiopathic arthritis. *Rheumatology International*, 2010, 30 (11), pp.1529-1532. 10.1007/s00296-010-1465-7 . hal-00581011

HAL Id: hal-00581011

<https://hal.science/hal-00581011>

Submitted on 30 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The turnover of synovial T cells in persistent oligoarticular Juvenile Idiopathic Arthritis is higher than in T cells in the peripheral blood

Juergen Brunner^{1,3}, Martin Herrmann², Markus Metzler³, Udo Gaipl⁷, Gert Reuter⁴
Johannes-Peter Haas^{5,6}

¹ Department of Pediatrics, Innsbruck Medical School, Anichstraße 35, 6020 Innsbruck, Austria

² Institute for Clinical Immunology, Department of Medicine III, Friedrich-Alexander University Erlangen-Nuremberg, Germany

³ Department of Pediatrics, Friedrich-Alexander University Erlangen-Nuremberg, Germany

⁴ Kinderrheumatologische Schwerpunktpraxis, Adalbertstr. 3, 90441 Nürnberg, Germany

⁵ Department of Pediatrics, Div. of General Pediatrics & Pediatric Rheumatology, University of Greifswald, Greifswald, Germany

⁶ German Centre for Rheumatology in Children and Adolescents, Garmisch-Partenkirchen, Germany

⁷ Department of Radiation Oncology, University Hospital of Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Germany

Corresponding author and author responsible for reprints:

Juergen Brunner M.D., Mag., MSc.

Department of Pediatrics, Medical University Innsbruck

Anichstraße 35, 6020 Innsbruck, Austria

Tel: +43-512-504-23500

Fax: +43-512-504-25450

email: juergen.brunner@uki.at

Running title: Cell cycle of T cells in JIA

ABSTRACT

Introduction: Juvenile idiopathic arthritis (JIA) summarizes a group of inflammatory diseases of childhood. The etiology remains still unclear. In JIA T cells have been demonstrated to play key roles in the pathogenesis. T-cell proliferation in JIA may be different in the peripheral blood (PB) and the synovial fluid (SF). The aim of this study is to demonstrate the turnover of T-cells in the PB and SF of patients with persistent oligoarticular JIA (oJIA) compared to controls.

PATIENTS AND METHODS

Matched pairs of samples were investigated derived from PB and synovial fluid SF of 9 patients with persistent oJIA. The cells from PB and SF were determined by flow cytometry.

RESULTS

The majority of the PBMC and IAMC were in phase G0/G1, with fewer than 1% in S phase. In the SF the percentage of cells in the S phase is higher than in the PB. The percentage of cells in the S phase in SF is equal to the result in the control group. In conclusion the turnover of synovial T- cells in persistent oJIA is higher than in the PB.

Word count:

Abstract: 182

Text: 823

Key words: cell cycle; juvenile idiopathic arthritis; S-phase; proliferation

INTRODUCTION

Juvenile idiopathic arthritis (JIA) summarizes a group of phenotypically heterogeneous chronic inflammatory diseases of childhood involving joints as well as extraarticular tissues. There are seven subtypes of JIA defined according to clinical classification criteria (9;10). Genetic factors, especially certain HLA-alleles have been shown to contribute to the susceptibility and have been additionally proposed to be involved in the pathogenesis of JIA (6). It has been supposed that HLA-alleles might present certain peptides thus shaping an autoreactive T-cell repertoire. The trimolecular complex of the MHC-molecule, the presented peptide and the T cell receptor (TCR) is one of the most important molecular interactions in the activation cascade leading to specific immunity (5;18). Stimulation with an antigen via the HLA presenting cell leads to stimulation of T-cells carrying a TCR specific for the peptide/HLA combination. These T cells will subsequently proceed to expansion (1;16). In JIA T cells have been demonstrated to play key roles, both for induction and maintenance of the chronic inflammation (4;17). Diminished mechanisms of T-cell homeostasis and premature aging of the immune system may play a role in the pathogenesis and perpetuation of chronic inflammation in JIA (11-13). T-cells in the peripheral blood (PB) and the synovial fluid (SF) of patients with rheumatoid arthritis (RA) have a higher proportion of actively synthesizing DNA (S-phase) cells than the T-cells in controls (14). The aim of this study is to demonstrate the turnover of T-cells in the PB and SF of patients with persistent oligoarticular JIA (oJIA) compared to controls.

PATIENTS AND METHODS

Matched pairs of samples were investigated derived from PB and synovial fluid SF of 9 patients (3 males, 6 females, mean age 8.6 years) with persistent oJIA with active arthritis (table 1). Diagnosis of JIA was based on the revised ILAR criteria (9). Other causes of arthritis as infections or malignancy have been excluded. All patients diagnosed with persistent oJIA were seen at two tertiary care centers and underwent clinical standardized assessment (9). Joint punctions have been performed for diagnostic and/or therapeutic reasons exclusively. 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. The controls are from adult normal healthy donors (NHD).

Laboratory analyses included blood cell count and C-reactive protein. All results were in normal range. All patients were treated with non steroidal anti-inflammatory drugs (NSAID) and/or methotrexate.

Peripheral blood mononuclear cells (PBMC) from peripheral blood (5–10ml, heparinized blood) and intraarticular mononuclear cells (IAMC) from SF (10ml anticoagulated with heparine) were prepared according to standard procedures (Lymphoprep, Nycomed Pharma AS, Asker, Norway). SF was filtered through a 100-nm nylon filter and centrifuged. The cell pellet was resuspended in RPMI- 1640 medium. Cell-cycle analysis on CD3+ T-cells was performed by propidium iodid staining. Immunofluorescence was determined by flow cytometry (Beckman-Coulter EPICS Elite ESP Sorter, Canada). Dead cells were excluded by gating on forward/side scatter and T cells were selected by gating CD3+ cells. 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).

RESULTS

The majority of the PBMC and IAMC were in phase G0/G1, with fewer than 1% in S phase (figure 1). In the SF the percentage of cells in the S phase is higher than in the PB (in promille) ($p < 0.05$) of oJIA patients and NHD (table 2). The percentage of cells in the S phase in SF is equal to the result in the control group. The percentage of cells in the G2 phase is higher in the PB of patients with persistent oJIA than in NHD ($p > < 0.0936$).

DISCUSSION

This is the first study investigating the cell cycle analysis of T-cells in the PB and SF of patients with persistent oJIA. The turnover of T-cells in the SF is higher than in the PB of oJIA patients ($p < 0.05$) and similar to the turnover of T-cells in NHD. Therefore a local proliferation in the SF may be considered. Other results were contradictory: T-cell proliferation was no greater in SF than in PB (2;3). Regarding the observation of expression of activation markers the findings suggest that SF T-cells in JIA are activated without proliferation (3). In RA the PB and SF has a higher proportion of S-phase cells compared to controls (8;14). However, regarding our own results, it may be possible, that small numbers of SF T-cells are driving an intraarticular inflammatory process due to an expansion of antigen-specific T cells within the joint (15). The proliferation in the PB in oJIA is reduced. Medical treatment may decrease the percentage of S-phase cells (7). In conclusion the turnover of synovial T- cells in persistent oJIA is higher than in PB. Considering the small number of patients, further studies are necessary to correlate cell cycle analysis with T-cell activation markers.

ACKNOWLEDGEMENT

This work was supported by the Federal Ministry of Education and Research (BMBF) and the Interdisciplinary Center for Clinical research (IZKF) at the University Hospital of the University of Erlangen-Nuremberg.

The authors declare that they have no conflict of interest.

REFERENCES

1. Bakakos,P., Pickard,C., Wong,W.M., Ayre,K.R., Madden,J., Frew,A.J., Hodges,E., Cawley,M.I., and Smith,J.L. (2002): Simultaneous analysis of T cell clonality and cytokine production in rheumatoid arthritis using three-colour flow cytometry. *Clin Exp.Immunol.*, 129:370-378.
2. Bergroth,V., Konttinen,Y.T., Pelkonen,P., Haapala,M., Haapasaari,J., Nordstrom,D., Kunnamo,I., and Friman,C. (1988): Synovial fluid lymphocytes in different subtypes of juvenile rheumatoid arthritis. *Arthritis Rheum*, 31:780-783.
3. Black,A.P., Bhayani,H., Ryder,C.A., Gardner-Medwin,J.M., and Southwood,T.R. (2002): T-cell activation without proliferation in juvenile idiopathic arthritis. *Arthritis Res*, 4:177-183.
4. Chini,L., Bardare,M., Cancrini,C., Angelini,F., Mancina,L., Cortis,E., Finocchi,A., Riccardi,C., and Rossi,P. (2002): Evidence of clonotypic pattern of T-cell repertoire in synovial fluid of children with juvenile rheumatoid arthritis at the onset of the disease. *Scand.J Immunol.*, 56:512-517.
5. Haas,J.P., Andreas,A., Rutkowski,B., Brunner,H., Keller,E., Hoza,J., Havelka,S., Sierp,G., and Albert,E.D. (1991): A model for the role of HLA-DQ molecules in the pathogenesis of juvenile chronic arthritis. *Rheumatol.Int.*, 11:191-197.
6. Haas,J.P., Nevinny-Stickel,C., Schoenwald,U., Truckenbrodt,H., Suschke,J., and Albert,E.D. (1994): Susceptible and protective major histocompatibility complex class II alleles in early-onset pauciarticular juvenile chronic arthritis. *Hum.Immunol.*, 41:225-233.

7. Julis,I., Skrivankova,B., and Trnavsky,K. (1986): DNA microfluorometry of synovial effusion cells in rheumatic diseases. *Clin Rheumatol*, 5:475-481.
8. Nordstrom,D., Konttinen,Y.T., Bergroth,V., Segerberg-Konttinen,M., and Santavirta,S. (1987): T cell subsets in the blast cell population in phytohemagglutinin-stimulated peripheral blood in vitro and in rheumatoid arthritis synovial fluid in vivo. *Rheumatol Int*, 7:67-70.
9. Petty,R.E., Southwood,T.R., Baum,J., Bhattay,E., Glass,D.N., Manners,P., Maldonado-Cocco,J., Suarez-Almazor,M., Orozco-Alcala,J., and Prieur,A.M. (1998): Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol.*, 25:1991-1994.
10. Prahalad,S. (2004): Genetics of juvenile idiopathic arthritis: an update. *Curr.Opin.Rheumatol.*, 16:588-594.
11. Prelog,M., Schwarzenbrunner,N., Sailer-Hock,M., Kern,H., Klein-Franke,A., Ausserlechner,M.J., Koppelstaetter,C., Brunner,A., Duftner,C., Dejaco,C., Strasak,A.M., Muller,T., Zimmerhackl,L.B., and Brunner,J. (2008): Premature aging of the immune system in children with juvenile idiopathic arthritis. *Arthritis Rheum.*, 58:2153-2162.
12. Prelog,M., Schwarzenbrunner,N., Sailer-Hoeck,M., Kern,H., Koppelstaetter,C., Wurznner,R., Zimmerhackl,L.B., and Brunner,J. (2008): Indications for a disturbed peripheral T-cell homeostasis in juvenile idiopathic arthritis (JIA): absent expansion of CD28 T-cells and no decrease of naive T-cells in cytomegalovirus-positive patients with JIA. *J Rheumatol*, 35:520-527.
13. Prelog,M., Schwarzenbrunner,N., Tengge,E., Sailer-Hock,M., Kern,H., Zimmerhackl,L.B., and Brunner,J. (2008): Quantitative alterations of

CD8(+) T cells in juvenile idiopathic arthritis patients in remission.
Clin.Rheumatol., 28:385-389.

14. Russell,A.S. (1990): Activated lymphocytes in the peripheral blood of patients with rheumatoid arthritis. J Rheumatol, 17:589-596.
15. Sieper,J., Braun,J., Wu,P., and Kingsley,G. (1993): T cells are responsible for the enhanced synovial cellular immune response to triggering antigen in reactive arthritis. Clin Exp Immunol, 91:96-102.
16. Teyton,L., Apostolopoulos,V., Cantu,C., III, Celia,H., Mallet-Designe,V., Stefanko,R., Stramann,T., and Wallace,M. (2000): Function and dysfunction of T cell receptor: structural studies. Immunol.Res., 21:325-330.
17. Thompson,S.D., Grom,A.A., Bailey,S., Luyrink,L., Giannini,E.H., Murray,K., Passo,M.H., Lovell,D.J., Choi,E., and Glass,D.N. (1995): Patterns of T lymphocyte clonal expansion in HLA-typed patients with juvenile rheumatoid arthritis. J Rheumatol., 22:1356-1364.
18. van der Helm-van Mil AH, Wesoly,J.Z., and Huizinga,T.W. (2005): Understanding the genetic contribution to rheumatoid arthritis. Curr.Opin.Rheumatol., 17:299-304.

FIGURE LEGENDS

Figure 1: Cell cycle analysis of intraarticular mononuclear cells (IAMC) from a patient with persistent oligoarticular juvenile idiopathic arthritis (oJIA). Most of the T-cells (B) are in G0/G1 phase (96.9%). 0.49% (A) are apoptotic, 0.76% (C) are in the S-phase and 1.1.0 % (D) are in the G2 phase.

Table 1: Characteristics of the investigated patients with persistent oligoarticular juvenile idiopathic arthritis (oJIA). Most of the patients had antinuclear antibodies (ANAs).

no	sex	age (y)	diagnosis	ANA	uveitis	medication
1	f	2	oJIA	positive	no	NSAID
2	m	12	oJIA	positive	no	NSAID
3	m	5	oJIA	negative	no	NSAID
4	f	4	oJIA	positive	no	NSAID
5	f	8	oJIA	positive	no	NSAID
6	f	16	oJIA	positive	no	NSAID
7	m	12	oJIA	positive	no	NSAID
8	f	10	oJIA	positive	no	NSAID, MTX
9	f	8	oJIA	positive	no	NSAID

Table 2: Cell cycle analysis of T cells of the peripheral blood (PB) and from the synovial fluid (SF) of patients with persistent oligoarticular juvenile idiopathic arthritis (oJIA). Most of the T-cells are in G0/G1 phase. In the SF the percentage of cells in the S phase is higher than in the PB (in promille) ($p < 0.05$). The two numbers in bold mark the most relevant results.

Patient	sample	all events	apoptosis (cells)	apoptosis (o/oo)	S phase (cells)	S phase (o/oo)	G0/G1 phase (cells)	G0/G1 phase (o/oo)
1	PB	18315	100	5,48	113	6,19	17996	986,35
1	SF	25142	228	9,14	196	7,86	24370	976,99
1	SF	25552	125	4,98	193	7,69	24465	975,17
2	PB	40826	129	3,16	278	6,80	40303	986,54
2	SF	33540	43	1,28	65	1,94	33394	995,53
3	PB	33983	612	18,07	81	2,39	33081	976,85
3	SF	16072	353	21,81	102	6,30	15656	967,49
4	PB	15639	198	12,66	34	2,17	15372	982,80
4	SF	21863	209	9,56	214	9,79	21389	978,10
5	PB	24544	902	36,87	126	5,15	23381	955,65
5	SF	4710	269	59,19	15	3,30	4254	935,97
6	PB	18042	408	22,52	37	2,04	17629	973,23
6	SF	17158	183	10,64	97	5,64	16880	981,34
7	PB	13869	386	27,57	50	3,57	13523	965,93
7	SF	24811	322	12,95	232	9,33	24246	975,11
7	SF	23035	138	6,00	75	3,26	22728	988,86
8	PB	21071	64	3,06	5	0,24	20285	970,71
8	SF	33128	290	8,94	89	2,74	31811	980,28
9	PB	21411	426	20,84	28	1,37	19479	952,98
9	SF	23840	721	31,76	103	4,54	21115	930,01
Mean	PB	23077	358	16,69	83,56	3,32	22338	972,33
Mean	SF	22622	261	16,02	125,55	5,67	21846	971,35
Mean	NHD	19546	456	24,40	108	7,72	18944	966,00