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No proinflammatory signature in CD34+ hematopoietic progenitor cells in multiple sclerosis patients

A Lutterotti[1,2], I Jelčić[1,3], C Schulze[4], S Schippling[1], P Breiden[1], B Mazzanti[5], S Reinhardt[1], M DiGioia[5], A Repice[6], L Massacesi[6], A Sputtek[7], G Salinas-Riester[8], N Kroeger[9], M Sospedra[1], R Saccardi[5], A Zander[9] and R Martin[1,3]

Abstract

Autologous hematopoietic stem cell transplantation (aHSCT) has been used as a therapeutic approach in multiple sclerosis (MS). However, it is still unclear if the immune system that emerges from autologous CD34+ hematopoietic progenitor cells (HPC) of MS patients is pre-conditioned to re-develop the proinflammatory phenotype. The objective of this article is to compare the whole genome gene and microRNA expression signature in CD34+ HPC of MS patients and healthy donors (HD). CD34+ HPC were isolated from peripheral blood of eight MS patients and five HD and analyzed by whole genome gene expression and microRNA expression microarray. Among the differentially expressed genes (DEGs) only *TNNT1* reached statistical significance (logFC=3.1, $p<0.01$). The microRNA expression was not significantly different between MS patients and HD. We did not find significant alterations of gene expression or microRNA profiles in CD34+ HPCs of MS patients. Our results support the use of aHSCT for treatment of MS.

Keywords

multiple sclerosis, hematopoietic stem cell transplantation, gene expression

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Introduction

Intense immunosuppression followed by autologous hematopoietic stem cell transplantation (aHSCT) is a potential treatment for patients suffering from aggressive multiple sclerosis (MS).[1] aHSCT is able to induce a long-lasting remission of inflammatory disease activity, which can persist years beyond complete immune reconstitution. The rationale for aHSCT in MS is based on the concept that lympho-/myeloablative conditioning eliminates pathogenic autoreactive immune cells and facilitates the regeneration of a new and tolerant immune system from CD34+ hematopoietic progenitor cells (HPC). In fact, thorough analysis of the T cell repertoire in the regenerating immune system after aHSCT in MS supports that a new and antigen-naïve T cell repertoire develops from the HPC compartment via thymic regeneration.[2] To date, it remains unresolved whether autoimmunity in MS is merely a consequence of loss of peripheral immune tolerance or whether it results from immune dysregulation, which is already predetermined in HPC. To

[1]Institute of Neuroimmunology and Clinical MS Research, Center for Molecular Neurobiology Hamburg, Germany.
[2]Clinical Department of Neurology, Innsbruck Medical University, Austria.
[3]Department of Neurology, University Hospital Zurich, Switzerland.
[4]Systems Biology and Protein-Protein Interaction, Center for Molecular Neurobiology Hamburg, Germany.
[5]Department of Haematology, Careggi University Hospital, Italy.
[6]Department of Neurological Sciences, University of Florence, Italy.
[7]Institute of Transfusion Medicine, University Medical Center Hamburg Eppendorf, Germany.
[8]DNA Microarray Facility, University of Medicine Göttingen, Germany.
[9]Center for Stem Cell Transplantation, University Medical Center Hamburg Eppendorf, Germany.

Corresponding author:
Andreas Lutterotti, Clinical Department of Neurology, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck, Austria
Email: andreas.lutterotti@i-med.ac.at

A.L., I.J. and C.S. contributed equally to the study.

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approach this key point we compared the global gene- and miRNA expression profiles of CD34+ and CD34- cells collected from MS patients and healthy donors (HD).

Patients and methods

Patients and controls

MS patients (n=8) with relapsing–remitting (RRMS) or secondary-progressive (SPMS) disease (mean disease duration 10 years, range 6–16 years) were treated with aHSCT at the University of Hamburg, Germany (four female SPMS) and the Haematology Unit, Careggi Hospital of Florence, Italy (two male RRMS and two female SPMS). All patients had previously received immunomodulatory and/or immunosuppressive therapy. Control HPC samples were obtained from five age-matched HD (three female). All patients provided written informed consent and all study protocols were in accordance with the Declaration of Helsinki and approved by Institutional Review Boards at each centre.

Mobilization and collection of CD34+ cells

Before collecting HPC from peripheral blood by leukocytapheresis, the Hamburg MS cohort and the five HD were mobilized with subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) analogue (2x5µg/kg/day) for 5–8 days. The Florence cohort was mobilized with intravenous cyclophosphamide (Cy, 4g/m^2) and G-CSF (5µg/kg/day) until cell harvest by leukocytapheresis. Cell collections were frozen in liquid nitrogen according to standard procedures.[3,4] All samples were thawed and processed at one centre by a standardized protocol and CD34+ HPC purified by magnetic bead separation using the autoMACS system (Miltenyi). The control samples consisted of the remaining CD34- negative cell fraction after magnetic bead separation, i.e. a population of peripheral blood mononuclear cells. Purity and viability of CD34+ cells were analyzed by FACS and revealed a mean of 84.8% (range 73.5 – 89.7%) viable CD34+ cells. There was no difference in the purity or viability of cells between MS patients and HD (see supplemental methods).

Microarray analysis

Whole genome gene expression was analyzed with the Human 4x44K Design Array (Agilent-Technologies). Differentially expressed genes (DEGs) of interest were confirmed by quantitative rtPCR. miRNA profiling was performed with the Human miRNA Array V2.0 (Agilent-Technologies). The microarray data were generated conforming to the MIAME guidelines and are deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27694).

Statistics and bioinformatics

Standard microarray analysis methods were used for processing intensity data and normalization (see supplemental methods).[5] Individual genes were considered differentially expressed above a fold-change of 1.7 (logFC>0.7). P-values were corrected for multiple testing.[6] miRNA data were analyzed in an analogous way.

Results

Gene expression analysis

Principal component analysis (PCA) confirmed separation between CD34+ and CD34- samples and showed a clear clustering of CD34+ cells according to the mobilization regimen

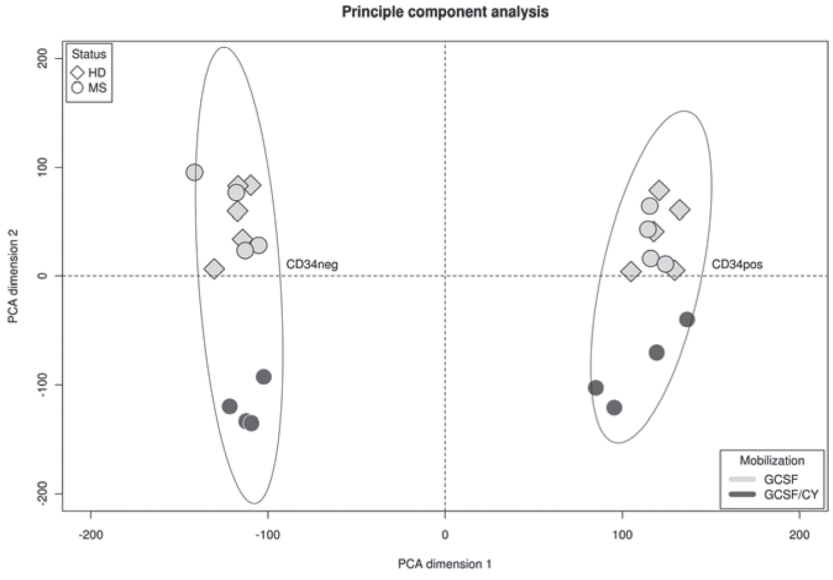

Figure 1. Principal component analysis (PCA) of CD34 positive cells and CD34 negative cells according to mobilization regimen. PCA of CD34 negative and CD34 positive cells from G-CSF treated (light grey) and G-CSF/Cy treated (dark grey) MS patients (circle) and G-CSF treated healthy donors (rhombus) shows a clear separation of samples according to the mobilization regimen.

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(Cy/G-CSF versus G-CSF; Figure 1). Accordingly, we found 2801 DEGs in CD34+ (adj.*p*-value≤0.05) and 9440 DEGs in CD34- (adj *p*-value≤0.05) cells comparing MS patients mobilized with G-CSF only or Cy/G-CSF, respectively.

Comparing DEGs in CD34+ HPC of MS patients and HD, both mobilized with G-CSF only, we found 297 DEGs (logFC>0.7), but the TNNT1 gene was the only DEG with statistical significance after Benjamini-Hochberg correction (logFC=3.1, adj.p<0.01; Table 1).

Comparing CD34- cells between MS and HD we found 167 DEGs (logFC>0.7), however none reached statistical significance.

miRNA expression analysis

miRNA expression was analyzed in samples obtained from MS patients mobilized with G-CSF only and HD mobilized with G-CSF only. None of the miRNA showed statistically significant differential expression levels comparing MS patients and HD mobilized with G-CSF only.

Discussion

The immunologic rationale for aHSCT as treatment for autoimmune diseases like MS is being discussed intensively

Table 1. Ten most up- and down-regulated genes in CD34+ HPCs comparing MS patients and healthy donors.

Gene symbol	Description	logFC	*p*-value[1]	Function
Upregulated				
TNNT1	Troponin T type 1 (skeletal, slow)	3.197	0.0000001[2]	Subunit of troponin, striated muscle contraction
FOXE1	Forkhead box E1 (thyroid transcription factor 2)	2.269	0.02534	Thyroid transcription factor
HLA-DQB1	Major histocompatibility complex class II. DQ beta	1.922	0.00560	HLA class II beta chain, expressed in antigen presenting cells
SOX17	SRY (sex determining region Y)-box 17	1.869	0.03167	Transcription factor, determination of the cell fate
GPR141	G protein-coupled receptor 141	1.780	0.11199	Rhodopsin family of G protein-coupled receptors
NEUROG1	Neurogenin 1	1.771	0.05177	Promotes neurogenesis, inhibits astrocyte differentiation
LOC647121	Embigin homolog (mouse) pseudogene	1.696	0.02507	Not known
TNXB	Tenascin XB	1.587	0.06001	Extracellular matrix glycoprotein, anti-adhesive effect
DKFZP434I0714	Hypothetical protein DKFZ-P434I0714	1.574	0.04861	Not known
ZSCAN10	Zinc finger and SCAN domain containing 10	1.568	0.07729	Transcription factor, metal ion binding
Downregulated				
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3	–2.454	0.14760	Putative RNA helicases
PRSS21	Protease.serine. 21 (testisin)	–2.209	0.00003	Cell-surface anchored serine protease
CTSG	Cathepsin G	–1.983	0.04575	Member of the peptidase S1 protein family, neutrophil granulocytes
MPO	Myeloperoxidase	–1.909	0.00095	Neutrophil azurophilic granules, microbicidal activity
FAM78A	Family with sequence similarity 78.member A	–1.844	0.06178	Not known
EIF1AY	Eukaryotic translation initiation factor 1A	–1.695	0.09977	Eukaryotic translation initiation factor
JARID1D	Jumonji. AT rich interactive domain 1D	–1.692	0.06539	Protein containing zinc finger domains
CYorf15A	Chromosome Y open reading frame 15A	–1.580	0.09665	Not known
ELA2	Elastase 2.neutrophil	–1.516	0.00429	Serine protease, neutrophil granulocytes
DNTT	Deoxynucleotidyltransferase. terminal	–1.428	0.00428	Template-independent DNA polymerase

HPCs: hematopoietic progenitor cells; logFC: log fold change; [1]: *p*-value (not adjusted); [2]: only gene which maintained statistical significance after correction (Benjamini-Hochberg).

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among basic and clinical immunologists in recent years.[2,7,8] A key issue has been the question whether replacement of the autoreactive immune system by autologous HPC is able to stop the autoimmune process for long or forever, or alternatively whether the autoaggressive immunity will rebound after hematologic reconstitution. If the latter occurred it would indicate that the autoimmune process is pre-programmed in HPCs of genetically predisposed individuals rather than evolving at the stage of mature T cells and in the peripheral immune system. In this study we approached this question by comparing the gene expression profile of CD34+ HPCs collected from MS patients before autologous transplantation with CD34+ HPCs or from HDs. To the best of our knowledge, this is the first study to analyze the gene expression profile of CD34+ HPC in an autoimmune disease.

The results of this study support the view that HPC of MS patients are not pre-conditioned towards autoimmunity. We did not find significant alteration in the gene expression profile of CD34+ HPC in MS. Only one DEG (*TNNT1)* maintained statistical significance after correction for multiple comparisons (Table 1). *TNNT1* encodes a subunit of troponins involved in contraction of slow skeletal muscle. Of note, the *TNNT1* gene is expressed on chromosome 19q13, which carries predisposing loci for several autoimmune diseases, but with conflicting results in MS.[9–11] A recent genome-wide association study did not find SNPs (single nucleotide polymorphism) associated with MS in the *TNNT1* gene.[12]

Comparison of miRNA expression profiles of CD34+ HPC between MS and HD did not reveal statistically significant differences, thereby corroborating our DEG results lacking substantial alterations in CD34+ cells in MS. There were no statistically significant DEGs in CD34- cells comparing MS and HD. The interpretation of our results must consider that the mobilization regimen with G-CSF provides a strong stimulus to the peripheral immune compartments and the stem cell niche and might thereby overshadow more subtle differences in the gene expression pattern of CD34- and CD34+ cells. Currently, experts recommend that the mobilization regimen for HSCT in MS should include G-CSF and cyclophosphamide, which precludes any comparison with HD. Since the mobilization regimen clearly influenced gene expression and HD are always mobilized by G-CSF only, our patient cohort provided a unique opportunity to directly compare the gene and miRNA expression profile of highly purified CD34+ cells from MS patients with HD. Consistent with our results, it has been shown that both the gene and miRNA expression differ depending on the stem-cell source and the mobilization regimen used.[13–15] Studies analyzing gene and miRNA expression in hematopoiesis or hematological malignancies mainly used HPC obtained by bone marrow aspiration or from in-vitro cultured cells, precluding a direct comparison with our results. A caveat in the interpretation of our study is the small number of samples, which leaves the possibility of a false negative result.

In summary, we did not find significant alterations of gene expression or miRNA profiles in CD34+ HPCs of MS patients. Thus, we provide evidence that the immune deviation seen in the peripheral immune system in MS patients is probably not at the CD34+ precursor cell stage. One must consider that the immune changes seen in MS may represent a secondary response to a primary CNS pathology. Nevertheless, we feel that the lack of significant alterations of gene expression or miRNA profiles in CD34+ HPCs of MS patients supports the use of autologous HPC for HSCT in MS.

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Conflict of interest statement

All authors declare no conflict of interests.

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