Journal of Neuroimmunology 299 (2016) 158–163

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Early hematopoiesis in multiple sclerosis patients

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ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form 17 August 2016
Accepted 7 September 2016

Keywords:
Multiple sclerosis
B cell
Precursor
NKT cell
Bone marrow
FACS

ABSTRACT

Contemporary evidence supports that MS immunopathology starts in the peripheral lymphatic system. However, the site and character of crucial initiating events are unknown. We examined subsets of the first stages of blood cells in the bone marrow of 9 MS patients and 11 neurologically healthy controls using FACS analysis. The proportion of natural killer T cells was lower ($P = 0.045$) in the bone marrow of MS patients, but proportions of hematogenous stem cells, myeloblasts, and B cell precursor subsets in the bone marrow did not differ between MS patients and controls. In this pilot study with a limited number of samples we found no deviation of the early B cell lineage in bone marrow from MS patients.

1. Introduction

MS is a chronic inflammatory disorder characterized by a long preclinical stage and a lifelong developing autoimmune process in the central nervous system, mainly targeting myelin. A complex of genetic and epidemiological risk factors and interactions are established. However, pivotal events turning the balance towards immunopathology are unknown, and new hypotheses on their site and character continue to be presented. They include influence from metabolites induced by gut microbiota (Rothhammer et al., 2016) and priming of lymphocytes by modified proteins in the lungs (Hedstrom et al., 2013, Odoardi et al., 2012). A few unconfirmed studies suggested a role for the bone marrow (BM) as a reservoir for latent viruses in MS (Fredrikson et al., 1989, Goswami et al., 1984, Kam-Hansen et al., 1988, Mitchell et al., 1978) (see Fig. 1).

Supported by analogy with experimental autoimmune encephalomyelitis and extensive immunohistological data, multiple sclerosis (MS) is considered a T cell–driven disease (Carbajal et al., 2015). Deviations in the proportion of T cell and natural killer (NKT) cell subsets in MS patients have been reported (Sellebjerg et al., 2012, Svenningsson et al., 1995), but B cells also have important roles in MS pathogenesis (Disanto et al., 2012) and are the only cells specifically targeted by effective MS pharmaceuticals (Sorensen and Blinkenberg 2016). The specificity of the majority of the oligoclonal IgGs in cerebrospinal fluid (CSF) and brain plaques (Mehta et al., 1981) of MS patients is unknown; however, there is a characteristic MS antiviral specificity in a minor IgG fraction in each patient (Hottenrott et al., 2015, Reiber et al., 1998). Distinctive features in MS neuropathology are structures in the brain similar to the immunoglobulin-secreting region of lymph nodes (Prineas 1979) and ectopic B cell follicles in the meninges (Howell et al., 2011, Serafini et al., 2004). According to contemporary hypotheses, these persistent B cell clones may arise through i) assistance from T follicular helper cells affected by primary immunopathology (Tangye et al., 2013) or pathological T cell–B cell interaction (Romme Christensen et al., 2013); ii) immortalization from infection with Epstein–Barr virus (EBV), which establishes a chronic infection in B cells and is an accepted risk factor for MS that is most pronounced when it manifests as infectious mononucleosis (Bagert 2009, Jons et al., 2015); and iii) genetic germline predisposition influencing the B cell lineage. The present pilot study focuses on the early B cell lineage in MS patients.

NKT cells are implicated in the control of autoimmunity (O'Keeffe et al., 2015), and they may be involved in the defense against viruses, including EBV (Chung et al., 2013). They originate from the bone marrow (BM) but mature in peripheral lymphoid tissues in adults, and their affinity to the BM in pathological conditions is unknown.

Several tissues including white blood cells, macrophages and possibly microglia in the brain are constantly rebuilt from the bone marrow.

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http://dx.doi.org/10.1016/j.jneuroim.2016.09.004
0165-5728/

We here hypothesize that disturbances in the early hematopoiesis, either genetic or caused by viral infections, are at the core of MS pathogenesis. Previous studies on BM in MS patients were small and their methods diverged. B-cell activation and immunoglobulin production was addressed (Fredrikson et al. 1991). Only one study has focused on the immunophenotypes of BM cells, showing a reduced cellular content that was thought to be induced by ongoing immunosuppressive treatment (Carrai et al., 2013).

We conducted an exploratory flow cytometry study of the BM of MS patients and neurologically healthy controls, examining basic mononuclear cell types and early lineage of BM cells in these two populations, particularly focusing on the proportions of stem cells, B cell precursors and NKT cells in MS.

2. Materials and methods

2.1. Patients and controls

We included nine MS patients (6 women, 3 men) with a median age of 51 years (range 41–56 years). Six had relapsing–remitting MS (RRMS), and the other three were in the secondary progressive phase (SPMS) (Table 1). The RRMS patients were clinically stationary during at least a year. One of the SPMS patients were in an active phase with a superimposed attack one year earlier, and MRI activity. Exclusion criteria were disease-modifying or experimental therapy during the last year and immunosuppressive therapy potentially associated with BM suppression. Glatiramer acetate (GA) therapy was ongoing in the RRMS cases, but the SPMS cases were untreated. Eleven neurologically healthy control patients (4 women, 7 men), scheduled for sternum-splitting thoracotomy for non-inflammatory cardiac disorders, were consecutively included. Median age for controls was 40 years (range 22–59 years). One cubic centimeter of BM was aspirated by crista puncture in patients and by sternal puncture immediately before sternotomy in controls (see Table 2b).

The study was approved by the Research Ethics Committee of Gothenburg 2010 (188-10).

2.2. Flow cytometry

Peripheral blood was collected in EDTA tubes and BM aspirate samples in saline-containing EDTA tubes at the Departments of Hematology (patients) and Cardiothoracic Surgery (controls) and transported to the Clinical Chemical Laboratory of the Sahlgrenska University Hospital. Within 24 h, erythrocytes were lysed using NH_4Cl, and cells were stained with the following monoclonal antibodies: CD105 and CD117 (Beckman Coulter, Marseille, France), CCR7 (R&D Systems, Oxon, UK), simultest (Cytognos, Salamanca, Spain), and the following from BD Biosciences (San José, CA): V450-conjugated anti-CD4 (clone RPA-T4), V450-conjugated anti-CD20 (clone L27), V500-conjugated anti-CD45 (clone HI30), simultest CD8 + lambda-FITC/CD56 + kappa-PE (ref CYT-SLPC-50), PerCP-Cy5,5–conjugated anti-CD5 (clone L17F12), PE-Cy7–conjugated anti-CD19 (clone SJ25C1), PE-Cy7–conjugated anti-TCR γ (clone 11F2), APC-conjugated anti-CD3 (clone SK7), APC-H7–conjugated CD38 (clone Hb7), V450-conjugated anti-HLA-DR (clone L243), FITC-conjugated anti-CD36 (clone CLB-IVC7), PE-conjugated anti-CD105 (clone 1G2), PerCP-Cy5,5–conjugated anti-CD34 (clone 8G12), PC7-conjugated anti-CD117 (clone 104D2D1), APC-conjugated anti-CD33 (clone P67,6), APC-H7–conjugated CD71 (clone M-A712), V450-conjugated anti-CD4 (clone RPA-T4), FITC-conjugated anti-CD27 (clone L128), PE-conjugated anti-CCR7 (clone 150,503), PerCP-Cy5,5–conjugated anti-smCD3 (clone SK7), PE-Cy7–conjugated anti-CD45R0 (clone UCHL1), APC-conjugated anti-CD45RA (clone HI100), APC-H7-conjugated CD8 (clone SK1), FITC-conjugated anti-CD5 (clone L17F12), and PE-Cy7–conjugated anti-HLA-DR (clone L243) (see Table 3b).

All cells were analyzed on a FACS Canto II (BD), with at least 30,000 acquired events per tube. Data files were analyzed using FacsDIVA software (BD). Myeloblasts were defined by low SSC, dim (D) expression of CD45, and expression of CD34 and CD117. More mature myeloid progenitors were defined by expression of SSC, CD45 + D, CD34-, and CD117+. Hematogones were defined by low SSC, CD19+, CD38+, and CD45 + D (Chantepie et al., 2013). Early hematogones (stage 1) were defined by very low SSC, CD45 + D, CD34+, and CD117-. The proportion of late hematogones (stages 2–3) was calculated by subtraction of the proportion of early hematogones from total hematogones. Mature B cells were defined by low SSC, CD45 + B, CD19+, and CD20+ and confirmed expression of light chains (kappa/lambda). T cells were defined as low SSC, CD45 + B, CD3+, and CD19- and further subdivided based on expression of CD4, CD8, CD27, CD45RA, CD45R0, and CD56, with NKT cells defined by expression of CD3+ and CD56+. Granulocytes and monocytes were defined based on CD45 and SSC properties and erythroblasts based on FSC, SSC, CD45−/+D expression, and concomitant expression of CD71 and CD36.

2.3. Statistical analysis

All statistical analyses were performed using SPSS (IBM Corp., Armonk, NY). Data are presented as medians and ranges. Differences in the proportions of different cell types between MS patients and control individuals were analyzed by the Mann–Whitney *U* test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Similarity of proportion of B cell precursors and mature B cells between patients and controls in BM and peripheral blood

The proportion of mature B cells did not differ between patients and controls, and the groups did not differ regarding the proportions of either total hematogones or stage 1 (early) or stages 2–3 (late) hematogones in the BM. In the B cell subsets, MS patients and controls did not differ in the proportion of CD5+ cells (Table 2a, b). MS patients and controls also did not differ regarding the kappa/lambda ratio in either blood or BM.

3.2. No deviation in MS patients regarding major leukocyte subsets in the BM or blood

When the frequencies of monocytes, granulocytes, B and T lymphocytes, and NK cells were assessed in BM and peripheral blood, patients and controls did not differ. Neither were there any differences between patients and controls in the proportion of myeloblasts (Tables 3a and 3b). The proportion of erythroblasts in peripheral blood was 1.70% in MS patients and 0.20% in the controls ($P = 0.038$), but no difference in this proportion was found in the BM.

3.3. T cell subsets in BM and peripheral blood

In agreement with previously published findings, we found no differences in proportions of CD4+ and CD8+ T cells or in subsets of these cells for CD27+, CD45R0, or CD45RA (Table 4a and 4b). In BM, the proportion of NKT cells was lower in the MS group than in controls (3.5% and 7.1%, respectively, $P = 0.046$) (Table 2a). A similar tendency was identified in blood but with no significant difference (2.4% and 7.5%, respectively, $P = 0.18$). When comparing only the RRMS patients with controls, the proportions of NKT cells were 2.1% and 7.1% in the BM, respectively ($P = 0.005$), and 1.6% and 7.5% in blood, respectively ($P = 0.027$).