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Brief Communication

Precursor B-cell development in bone marrow of Good syndrome patients

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A B S T R A C T

Good syndrome is an immunodeficiency presenting with thymoma, hypogammaglobulinemia and almost absent B cells. To investigate the origin of the B-cell lymphopenia in these patients, we studied B cell differentiation in the bone marrow of Good syndrome patients. We found very low numbers of precursor B cells in bone marrow of Good syndrome patients and a differentiation arrest after the pro-B-cell stage; this is different from other agammaglobulinemia patients with a defect in pre B-cell receptor signaling.

1. Introduction

Good syndrome (GS) [1] is a rare immunodeficiency presenting with thymoma, consisting of neoplastic thymic epithelial cells and non-neoplastic maturing thymocytes, hypogammaglobulinemia and almost absent B cells [2,3]. Both sexes are equally affected and age of onset is between 40 and 70 years. Patients suffer from severe recurrent infections with mainly encapsulated bacteria and opportunistic pathogens [1,3]. Several reports indicate a poor prognosis and survival in GS patients due to the high susceptibility for severe bacterial infections and because of autoimmunity [3]. Although GS pathogenesis is largely unclear, the severe reduction of peripheral B cells suggests impaired differentiation of B cell precursors (BCP) in bone marrow (BM) as previously reported in small series of GS patients [4,5].

2. Study design

2.1. Study patients and samples

In this study we analyzed the clinical and immunological phenotype of 9 GS patients. We included BM samples from 9 GS patients. Additionally, BM samples from 15 healthy donors (HD) (age > 10 years) and 19 patients with agammaglobulinemia with a defined inborn defect in the BCP maturation (X-linked agammaglobulinemia, μ-chain-, CD79- or BLNK deficiency) were included. These agammaglobulinemia patients all have a defect before the pre-BII cell stage because of defect in pre- B-cell receptor signaling. This study was performed according to the guidelines of the Medical Ethics Committee of the Erasmus MC and RadboudUMC (METC MEC-2013-026/NL40331.078.12) and was approved by the Karolinska Institutet Research Ethics Committee south 144/01.

2.2. Flow cytometry analysis

BCP maturation stages in BM samples were studied using flow cytometry, as previously described [6]. Only in 6 out of 9 patients precursor B-cells could be identified. The relative sizes of 4 different populations (ProB, PreB-I, PreB-II, Immature) were expressed as percentages of total BCP. Mature B cells were excluded from the calculations. Statistical analysis was performed with GraphPad Prism software (GraphPad Software, La Jolla, Ca); Mann-Whitney test was used.

3. Results and discussion

Clinical and immunological characteristics of the GS patients are shown in Table 1. All 9 GS patients (4 females, 5 males) had undergone thymectomy and 8 had histologically confirmed thymoma. The predominant infections were similar to what was previously described in GS patients [2,7]. Those comprise mainly respiratory tract infections and in some cases opportunistic infections were recorded. All patients receive immunoglobulin substitution therapy. One patient suffered

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Table 1
Clinical and immunological characteristics of the Good syndrome patients. Thymoma pathology was according to WHO Classification of Thymic Epithelial Tumors (1999).

Patient	Age/sex	Pathology thymoma	IgG levels (g/l) at diagnosis	IgA	IgM	Infections	Therapy/Thymectomy	Other morbidity/Specific symptoms
Patient 1	62/M	Type B2	4.8	0.14	< 0.30	Recurrent airway infections: haemophilus	Valaciclovir Chemotherapy: Cisplatinum/Etoposide IVIg[a]/Yes	Recurrent Herpes simplex Loss of CD36 on the monocytes Mannan-binding lectin deficiency Vena cava superior syndrome
Patient 2	65/M	Type AB	5.6	1.63	0,44	Recurrent airway infections: haemophilus	Radiation IVIg/Yes	
Patient 3	67/F	Type AB	0.24	< 0.06	0.05	Rarely airway infection	SCIg[b]/Yes	Chronic skin/mouth rash: GVH due to thymoma or lichen ruber. Kidney failure.
Patient 4	60/M	Type AB	2.7	< 0.10	< 0.10	Bronchitis 2–3 times/year Skin mycosis	SCIg/Yes	Bronchiectasies. Pure red cell anemia for two years after thymectomy
Patient 5	51/M	PAD not available	1.6	0.10	< 0.04	Bronchitis/pneumonia frequently Skin mycosis	SCIg/Yes	Bronchiectasies.
Patient 6	56/F	Type AB	4.4	0.5	< 0.04	Pneumonias before SCIg. Then rarely infections.	SCIg/Yes	Mastectomy due cancer mam. Bleeding disorder (mild). Bronchiectasies (minor).
Patient 7	73/F	Type AB	0.33	0.03	< 0.04	Upper and lower airway infections before SCIg. Reduced after treatment.	SCIg/Yes	Chronic bronchitis. Myasthenia gravis that disappeared after thymectomy.
Patient 8	46/F	Unknown	1.3	< 0.05	< 0.1	Recurrent upper airway	IVIg	Increased growth hormone Acromegalic appearance
Patient 9	71/M	Type A	2.0	0.50	0.31	CMV Pneumocystis carinii Persistent Noro virus Pneumonia: haemophilus	IVIg/Yes	Skin carcinoma, basalioma, hepatosplenomegaly, liver fibrosis, lymphadenopathy

[a] IVIg, intravenous immunoglobulin substitution therapy.
[b] SCIg, subcutaneous immunoglobulin substitution therapy.

from autoimmune pure red cell aplasia two years after thymectomy.

Concurrent peripheral blood analysis (Supplementary Table 1) in GS patients revealed a severe reduction of peripheral blood B cells, 5 patients had a mild reduction in T cells counts, 4 patients had reduced levels of $CD4^+$ T cells and 3 patients had lower $CD8^+$ counts (Supplementary Table 1).

The overall cellularity of the BM in GS patients was normal, white blood cell count and myeloid cells were not reduced in GS patients compared to HD. However, the median frequency of normoblasts were reduced in GS patients ($P < .05$). GS patients present a median of 3,9% of normoblast in comparison to HD with median frequency of normoblast of 11,5% (data not shown). The analysis of BM samples from GS patients showed a severe reduction in the frequency of BCP ($CD22^+$) but high frequencies of T cells ($CD3^+$) compared to HD ($P = .0015$; $P = .0057$ respectively) and agammaglobulinemia patients ($P = .0006$; $P = .0492$ respectively) (Fig. 1A).

GS patients had a significantly higher frequency of ProB cells ($CD19^-$ $CD34^+$ TdT^+ $cyIg\mu^-$) and PreB-I cells ($CD19^+$ TdT^+ $CD34^+$ $cyIg\mu^-$) compared to HD ($P = .0005$ and $P = .0005$ respectively) (Fig. 1B). However, relative frequencies of PreB-II cells ($CD19^+$ $CD34^-$ TdT^- $cyIg\mu^+$) and Immature cells ($CD19^+$ $cyIg\mu^+$ IgM^+ IgD^-) were significantly decreased in GS patients compared to HD ($P = .0005$ and $P = .0005$ respectively). Thus, there is a significantly reduced number of BCP in the BM of GS patients and the BCP that are present are mainly ProB cells and PreB-I cells. The age of the patients do not seem to be involved with the reduction of these populations, since there is no correlation between the age of the patients and the amount of ProB and PreB-I cells.

The composition of the BCP compartment in GS patients differs from the agammaglobulinemia patients (Fig. 1B). Agammaglobulinemia patients showed a relative expansion of ProB and PreB-I cells compared to HD ($P < .0001$ and $P < .0001$ respectively), combined with a significant reduction later in differentiation (Pre B-II and Immature cells $P = .0016$ and $P < .0001$ respectively) due to a defect in preB-cell receptor signaling, which precludes further differentiation.

GS and agammaglobulinemia patients showed a different type of arrest in BCP differentiation (Fig. 1C, D). In GS patients there was a trend towards a relative increase in mainly the ProB cells ($P = .0013$), together with a decrease in PreB-I cells compared to agammaglobulinemia patients ($P = .0170$). Also the immature B cell subsets are significantly reduced in BM from GS patients ($P = .0111$). The arrest in GS patients seems to occur after the ProB stage, whereas in agammaglobulinemia patients a differentiation arrest occurs after the Pre B–I stage.

GS has been compared to common variable immunodeficiency (CVID) previously. In both diseases BAFF-R and TACI mutations have been identified [8]. To our knowledge, this is the first comparison to patients with monogenic causes of agammaglobulinemia who also have a block in BCP development in bone marrow.

Previous studies on the T cell compartment of GS patients revealed frequent $CD4^+$ lymphopenia [7], a case with increasing γδ T cells [9] and a case presenting $CD8^+$ T cell large granular lymphocyte leukemia with an exhausted phenotype [10]. The clonal expansion of $CD8^+$ T lymphocytes in bone marrow has been described and associated with B-lymphopoiesis deficiency. The authors hypothesized this was due to a $CD8^+$ T cell-driven immune response against BCP [11]. Other hypotheses have been proposed to explain GS pathogenicity, such autoimmune destruction of B cells mediated by autoantibodies [7]. Pathogenic antibodies are commonly present in GS patients, for example anti-acetyl choline receptor antibodies causing myasthenia gravis [2]. The aberrant thymic microenvironment may predispose for self-activation of thymocytes against autoantigens, and thus evoke a T-cell response against B cells. Additionally, murine models have shown a role for interferon-like cytokines such as Limitin, which is produced by bone marrow stromal cell line. This was hypothesized to be involved in BCP differentiation, promoting cell cycle arrest upon impaired differentiation [7]. Aberrancies in BM stromal environment could cause a maturation arrest of the BCP. To test this hypothesis, it would have been of

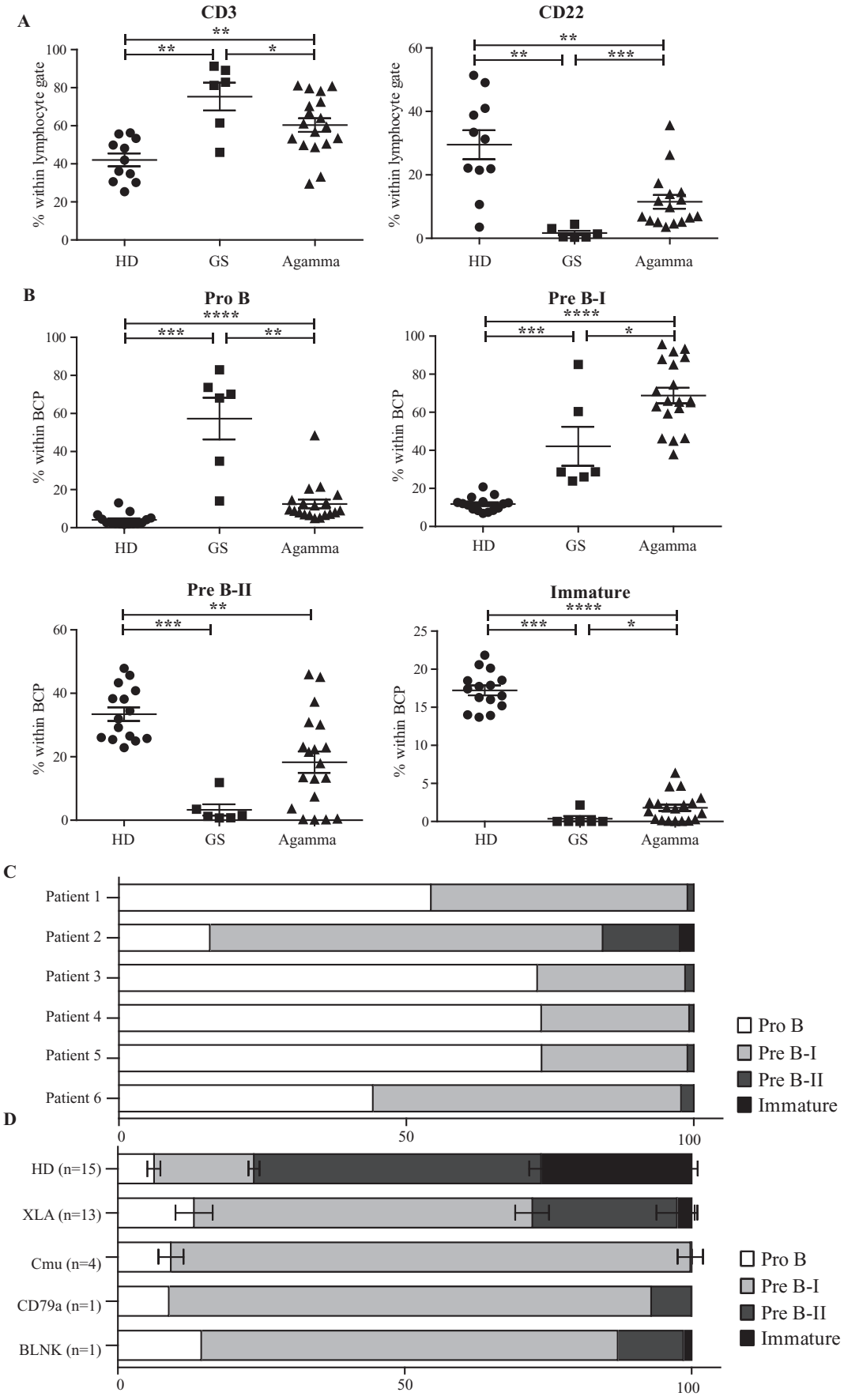

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Fig. 1. Immunophenotyping of BCP in BM of Healthy donors (HD) (n = 15), Good syndrome (GS) (n = 6) and agammaglobulinemia (n = 19; for subtypes see D panel) patients. A. Relative frequencies of $CD3^+$ T cells and CD22+ B cells in BM samples. $CD22^+$ B cells were CD3/D13/CD33/CD36/CD16/$CD56^-$). B. Relative frequencies of BCP populations: proB ($CD19^+$ cyIgμ$^-$), PreB-I ($CD19^+$ cyIgμ $^-$), PreB-II ($CD19^+$ cyIgμ $^+$$IgM^-$) and Immature B cells ($CD19^+$ cyIgμ $^+$ IgM^+ IgD^- $CD10^+$ $CD38^+$). C. Subset distribution of BCP subsets in BM for all individual GS patients. D. Subset distribution of BCP subsets in BM for HD (pooled) and agammaglobulinemia patients divided on genetic diagnosis.

great interest to compare the differentiation and proliferation of pro-B cells from HD and GS patients in a feeder-free culture system, as previously described by Kraus et al. [12].

In conclusion, this study demonstrates that the absence of peripheral blood B cells in GS patients is reflected in very low numbers of BCP in the bone marrow of these patients. The BCP that are present show an arrest in differentiation mainly after ProB stage, which is different from a selected series of patients with defined monogenic agammaglobulinemia, in whom a block is found after PreB-I stage, which is linked to the absence of pre-B cell receptor signaling.

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Authorship contribution

LdPM and MW performed research and analyzed and interpreted data. MvdB designed the study. MvdB, MvD, MvH and CIES conceived the study. LdPM, MW and MvdB wrote the paper.

Conflict-of-interest disclosure

All authors state no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2018.11.009.

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