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Regular Article

Imbalance between CD205 and CD80/CD86 in dendritic cells in patients with immune thrombocytopenia

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ABSTRACT

Introduction: CD205(DEC-205), a tolerance-associated receptor, is a member of the macrophage mannose receptor family of C-type lectin receptors. Antigen uptake via CD205 induces regulatory T cells, thereby regulating peripheral immune tolerance. However, the contribution of CD205 to autoimmune diseases has not been elucidated. Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by overdestruction of platelets. A previous study by the present authors found that CD205 expression in dendritic cells (DCs) was upregulated during induction of immune tolerance in patients with ITP.

Methods: CD205 expression in monocyte-derived DCs and spleens from patients with ITP was analysed prior to and after high-dose dexamethasone (HD-DXM) treatment. Expression of CD80, CD86 and HLA-DR was also analysed in order to identify and define the maturation status of the DCs more precisely.

Results: In patients with ITP, CD205 expression was found to be significantly decreased in DCs, and rare or absent in the border region of the spleen. However, the expression of CD80 and CD86 was increased in both monocyte-derived DCs and spleens in patients with ITP compared with controls. HD-DXM treatment may upregulate CD205 expression and downregulate CD80/CD86 expression, then rebalance the expression of CD205 and CD80/CD86 in DCs in patients with ITP.

Conclusion: Imbalance between CD205 and CD80/CD86 may contribute to the development of ITP. Therapies that aim to restore the balance between CD205 and CD80/CD86 may help to re-establish tolerance in patients with ITP.

Introduction

Immune thrombocytopenia (ITP) is an autoimmune disease characterized by overdestruction of platelets. Evidence exists for a variety of defects in dendritic cells (DCs), T cells and B cells in patients with ITP, but the causative factors that lead to loss of tolerance to platelets are still under investigation [1]. As essential antigen-presenting cells, DCs play a critical role in the initiation and maintenance of autoimmunity. In a previous study, the authors found that induced regulatory T cells (iTregs) could induce platelet tolerance through rendering DCs tolerogenic, because iTreg-modulated DCs fail to stimulate autoreactive T-cell proliferation [2]. Using microarray analysis, CD205(DEC-205) expression in DCs was shown to be upregulated during induction of tolerogenic DCs [2]. Thus, it was speculated that CD205 may be involved in the pathogenesis of ITP.

CD205 is one of the least understood members of the macrophage mannose receptor family of C-type lectin receptors [3,4], which can capture endogenous glycoproteins and contribute to immune tolerance. It is an important tolerance-associated receptor that is mainly expressed in DCs and upregulated during DC maturation [5,6]. Studies have shown that antigen uptake via CD205 allows more efficient delivery to the processing compartment, and induces T-cell unresponsiveness thereby regulating peripheral immune tolerance [7–11]. However, to the authors' knowledge, the contribution of CD205 to autoimmune diseases has not been reported. The authors have shown previously that CD205 expression in DCs is decreased significantly in both monocyte-derived DCs and spleens in patients with ITP. On the contrary, CD80/CD86 expression is increased in patients with ITP. High-dose dexamethasone (HD-DXM) could shift the balance between CD205 and CD80/CD86 towards higher expression of CD205 and lower expression of CD80 and CD86 in DCs.

<A> Materials and Methods

<B> *Patients and Controls*

Twenty-seven newly diagnosed patients with ITP were enrolled in this study and received HD-DXM 40 mg/day for 4 consecutive days as

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initial treatment (Table 1). The diagnosis of ITP was based on criteria reported previously [12]. The response to HD-DXM was evaluated as follows [12].

- A complete response was defined as a platelet count of $\geq 100 \times 10^9$/l.
- A response was defined as a platelet count of 30–100 $\times 10^9$/l and a doubling, or more, of the baseline count.
- No response was defined as a platelet count of $< 30 \times 10^9$/l, or less than a doubling of the baseline count.

Blood samples from 12 healthy donors were used as controls. Blood sampling was performed prior to and 2 weeks after HD-DXM initiation. Spleens were obtained from six patients with ITP and four healthy donors who underwent splenectomy due to traumatic injury. This study was approved by the Medical Ethical Committee of Qilu Hospital, Shandong University and was conducted in accordance with the Declaration of Helsinki.

<B> *Preparation of DCs*

CD14+ cells were negatively selected from peripheral blood mononuclear cells using micromagnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The purified CD14+ cells were incubated in RPMI 1640 culture medium with 10% fetal calf serum (Hyclone, Utah, USA), 1000 μ/ml granulocyte–macrophage colony stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN) and 1000 μ/ml interleukin-4 (R&D Systems) for 5 days. Lipopolysaccharides (LPS; 1 μg/ml; Sigma, St Louis, MO) were added to the cultures for an additional 2 days. In some experiments ($n = 6$), different concentrations of DXM (0 nmol/l, 10 nmol/l, 25 nmol/l, 50 nmol/l and 100 nmol/l) were added to the culture system during DC induction. On Day 7, the DCs were harvested for flow cytometry and quantitative polymerase chain reaction analysis.

<B> *Flow Cytometry*

Cell staining was performed using FITC- or PE-conjugated anti-CD205, anti-CD80, anti-CD86 and anti-HLA-DR (eBioscience, San Diego, CA) monoclonal antibodies. The same-species-isotype immunoglobulin G was used as an isotype control. Data acquisition was performed using a FACScalibur flow cytometer (BD Biosciences, San Diego, CA), and data were analysed using Cell Quest Pro software (BD Biosciences).

<B> *Quantitative Real-time Polymerase Chain Reaction Analysis*

Total RNA was extracted from DCs by TRIzol (Invitrogen, Carlsbad, CA). cDNA was synthesized using the PrimeScript RT reagent kit (Takara Biotechnology, Inc., Japan). PCR was performed with Power SYBRGreen PCR Master Mix (Takara) with denaturation at 95 °C for 5 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 10 s and 72 °C for 10 s. Melt curve analysis was performed following amplification. The primers selected were as follows: CD205, forward, 5'-AGTGGTGTTGGAAGATGTAGCA-3'; reverse, 5'-GTCCGCCCATGAGAACTAAG-3'; CD80, forward, 5'-GGGC ACATACGAGTGTGTTGT-3'; reverse, 5'-TCAGCTTTGACTGATAACGTCAC-

Table 1
Clinical characteristics and initial responses of ITP patients.

Patint	sex/age	Antiplatelet antibbody		Bleeding	Comorbidities	Platelet count(×10^9/L)		Previous treatment
No.	(year)	Anti-GP IIb/IIIa	Anti-GP Ib/IX	sympotoms		Pre-treatment	Post-treatment	
*1	M/41	+	-	PT	None	1	52	None
*2	M/19	+	-	EC EP	None	15	136	None
*3	FM/38	+	+	PT	None	2	64	None
*4	FM/40	+	-	EP	None	10	206	None
*5	FM/46	+	-	PT	None	2	27	None
*6	FM/39	-	+	PT EP	None	11	65	None
*7	M/60	+	-	PT	None	4	115	None
*8	M/35	+	+	EC EP	None	17	127	None
*9	FM/46	+	-	PT EC	None	2	154	None
*10	FM/16	-	+	PT EC	None	21	257	None
*11	M/26	+	-	EC	None	11	19	None
*12	M/59	+	-	EC	None	4	69	None
*13	M/49	-	+	PT	None	12	125	None
*14	FM/45	-	+	GUH	None	3	42	None
*15	M/57	+	-	PT	None	6	126	None
*16	FM/66	+	-	PT EP	None	5	47	None
*17	FM/48	+	+	EP GH GUH	None	1	65	None
*18	M/73	-	+	PT GH	None	3	12	None
*19	FM/33	+	-	GUH	None	9	100	None
*20	M/65	+	-	PT	None	5	234	None
*21	FM/48	+	-	PT	None	3	17	None
*22	FM/28	+	-	PT EC	None	8	13	None
*23	FM/46	-	+	PT GH	None	3	54	None
*24	FM/16	-	+	PT EC	None	21	155	None
*25	FM/25	+	-	EC	None	11	251	None
*26	M/77	+	+	PT EP	None	10	67	None
*27	FM/76	-	+	PT EC	None	2	32	None
28	FM/55	+	+	EC	None	21	207	Daily low-dose prednisone
29	FM/27	+	+	EP	None	6	312	Daily low-dose prednisone
30	FM/46	-	+	EC	None	4	63	Daily low-dose prednisone
31	M/49	+	-	PT EC	None	4	148	Daily low-dose prednisone
32	M/53	+	+	PT GH	None	8	325	Not available
33	M/43	+	+	PT EP	None	12	111	Daily low-dose prednisone

EC:ecchymosis, EP:epistaxis, GH:gingival hemorrhage, GUH:genitourinary hemorrhage, PT:petechia.
* indicates patient who were treated with HD-DXM.
indicates patient who received splenectomy.