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High Production of IL-18 by Dendritic Cells Induced by Sera from Patients with Primary Antibody Deficiency

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ABSTRACT

Predominantly antibody deficiencies are a category of primary immunodeficiency diseases, which consist of several rare disorders such as common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA). We evaluated the effects of CVID and XLA patients' sera as a source of microenviromental factors on maturation and function of monocyte-derived DCs.

Blood was collected from 10 CVID and 5 XLA patients before immunoglobulin replacement therapy and also from 8 healthy volunteers in order to obtain necessary sera for this study. Monocyte derived DCs were generated from blood cells obtained from healthy volunteers in the presence of GM-CSF, IL-4 and 10% serum concentrations from cases and controls. Immature DCs were incubated with monocyte conditioned medium (MCM) and TNF-α in order to generate mature DCs. Interleukin 18 (IL-18) production by CD40L-activated mature DCs was measured after 24 hours of culture *in vitro*.

IL-18 production by DCs generated in the presence of CVID and XLA patients' sera were 6.75±2.59 and 7.08±1.75 ng/ml, respectively, which were significantly higher than normal serum conditioned DCs (3.55±0.68) ng/ml.

These results suggest that the sera of patients with predominantly antibody deficiencies may contain soluble factor(s) that can induce a significant increase in IL-18 production by DCs.

Keywords: Common variable immunodeficiency; Dendritic cells; Interleukin-18; X-linked agammaglobulinemia

INTRODUCTION

Predominantly antibody deficiencies are a category of primary immunodeficiency diseases, which consist of several rare disorders such as common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA).[1-3] CVID is a heterogeneous group of disorders, characterized by defective antibody production with decrease in at least 2 immunoglobulin isotypes and an increase in susceptibility to recurrent pyogenic infections as well as autoimmune and neoplastic disorders.[1, 3-8] The number of circulating B

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cells is reduced or remain normal. B cells in these patients can proliferate and produce immunoglobulins *in vitro* if given appropriate T cell signals.[9] XLA or Btk deficiency is a relatively rare disease characterized by a profound B cell deficiency due to an arrest in B-lymphocyte development, resulting in severe hypogammaglobulinemia and recurrent infections; however, in these patients, T-cell function and numbers are normal. The clinical characteristics of XLA are recurrent pyogenic infections starting in infancy, and hypoplasia or atrophy of lymphoid tissues.[2,10,11]

The Th1 response could lead to impairment of antibody production by B cells in CVID patients.[4,12] It is speculated that B cells may not receive appropriate signals from T helper lymphocytes and abnormal interaction in germinal center may be involved in the pathogenesis of the disease.[13] Under physiologic conditions, humoral immune responses to T dependent antigens are initiated in the T cell area of secondary lymphoid organs, where naïve T cells physically interact with DCs and subsequently are stimulated. DCs are the most potent APCs, distinguished by their exceptional capability to prime naïve T cells and orchestrate the adaptive immune response.[14,15] In addition to T cell stimulation, DCs in particular follicular DCs regulate B-cell growth and immunoglobulin secretion/class switching and differentiation towards plasma cells.[16,17]

Interleukin-18 (IL-18), also known as interferon-gamma inducing factor, is a pleiotropic cytokine, which is mainly produced by APCs, especially DCs. This cytokine seems to play an important role in the development of T helper type 1 cells, similar to interleukin-12 (IL-12). Additionally, IL-18 is an IL-1 related proinflammatory cytokine which plays a pivotal role in systemic and local inflammation. Although IL-18 and IL-12 share the capacity to induce IFN-γ production by activated Th1 cells, their induction pathways seem to be independent. In addition, IL-18 which is capable of inducing 2-5 times more IFN-γ than optimal doses of IL-12 will augment more IFN-γ even in the presence of saturated amounts of IL-12.[18-20] IL-18R similar to IL-12R is selectively expressed on Th1 but not on Th2 cells and can therefore be considered as a cell surface marker to distinguish Th1 from Th2 cells.[21-23] There was an evidence for the association between increased expression of IL-12R and IL-18R on a subset of naïve T cells and granulomatous manifestations in CVID. These findings provide further evidence of a polarization towards a Th1 immune response in CVID.[24]

Although malfunctioning of DCs appears to be a prominent feature of CVID patients (17), it is unclear whether this property is related to the effect of CVID patient's micro environmental factors or which are inherently impaired. In the present study, we evaluated the effects of CVID and XLA patients' sera as a medium which may contain factors influencing differentiation, maturation and cytokine production by DCs. Association of CVID with a particular pattern of cytokine expression like elevated IL-18 that might lead to a polarized Th1 response, is also explored in the current investigation.

MATERIAL AND METHODS

Patients and Controls

In this study, 10 CVID and 5 XLA patients and 8 normal subjects (laboratory personnels) were evaluated. The patients were selected from a group of patients who had been referred to the Children Medical Center, Tehran University. The diagnosis of CVID and XLA was based on standard criteria, which has been introduced by the Expert Committee of International Union of Immunological Societies (IUIS) on Primary Immunodeficiency. [3] Btk mutations were detected in all XLA patients. [10] In all patients, the serum immunoglobulin levels (IgG, IgM and IgA) were measured by Nephelometry and B-cell and T-cell CD markers (CD19, CD3, CD4 and CD8) were assessed by flow cytometry. Following informed consent and approval of the local Ethics Committee, blood was collected from the patients before immunoglobulin replacement therapy.

Cell Isolation and Culture Conditions

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood after taking informed consent from healthy donors. Dendritic cells were generated from monocyte: In brief, immature DCs were prepared from 4×10^6 PBMCs isolated using lymphoprep 1.077±0.001g/ml (Axis-Shield, Oslo, Norway) and plated in flasks containing RPMI 1640 (GIBCO Life Technologies, Grand Island NY) supplemented with 10% human AB serum (Iranian Blood Transfusion Organization, Tehran, Iran), 2nM L-glutamine (GIBCO, Scotland, UK), 100 u/ml penicillin and 100 µg/ml streptomycin (GIBCO Invitrogen

Corporation, NY), and 10mmol/l HEPES (Merck, Germany), in a 5% CO2 incubator at 37˚C. After 2 h, nonadherent cells were removed by 3 gentle washings with medium. The remaining adherent monocytes were cultured in complete RPMI medium with 10% human serum, glutamine, and antibiotics in the presence of 500 IU/ml rhIL-4 and 1000IU/ml rhGM-CSF (both from BenderMedSystems GmbH. Austria). On day 3 half the medium, including all supplements, were replaced. After 5 days, nonadherent and loosely adherent cells were harvested, washed, and used for immuno-phenotyping as immature dendritic cells. Mature DCs were generated by adding 40ng/ml TNF-α (BenderMedSystems GmbH, Austria) and 30% MCM[25] for 2 additional days. After 7 days mature DCs were harvested, washed, and used for immunophenotyping and other experiments. Mature DCs with peripheral dendrites were seen at day 7. Cytospin staining slides of cultured cells were found to contain cells with typical figure of immature and mature DCs (Figure 1).

To examine the effect of patients and normal sera on the differentiation of DCs, 10% of serum from CVID and XLA patients was used in separated wells on day 0. The same concentration of serum was also employed in the maturation phase.

Monocyte Conditioned Medium (MCM)

MCM that is produced by culturing monocytes on immobilized human γ-globulin, appears to be a critical component of the maturation process.[26] MCM was prepared as previously described by Reddy et al.[26] with some modifications. Ig coated bacteriologic plates (100 mm, Falcon) were prepared by adding 10ml of 5mg/ml human gamma globulin (Baxter, Hyland Immuno); after 5 minutes, the plates were washed three times with sterile PBS. PBMCs (10×10^7) isolated from buffy coat by Ficoll-Hypaque were layered onto the Ig-coated bacteriologic plates for 1 hour in 10ml complete medium with 10%human AB serum. Nonadherent cells were washed away and discarded. Ig-adherent cells were incubated in fresh complete medium with 20% human AB serum at 37˚C for 24 hours. The medium was collected, centrifuged and the cell-free supernatant was passed through a 0.22 μm filter and frozen at -20˚C for later use.

Cytokine Production by Dendritic Cells

To evaluate the cytokine production by serum treated DCs, mature DCs (5×10^5/well/0.5ml) were stimulated with 1μg/ml CD40L (Alexis, Lausen, Switzerland) in 24 well plates and incubated at 37 °C, 5% CO2 incubator for 24 hrs and cell free supernatants were collected and stored at -70˚C for subsequent cytokine assays.[25]

Sandwich ELISA kit for IL-18 was purchased from BMS (BenderMedSystems, GmbH, Austria) company. Detection limits of the kit was 55 ng/ml.

Statistical Analysis

Statistical analyses were performed using analysis of variance (ANOVA) and a multiple comparison test of Tukey HSD test (as a post hoc test). Our results were expressed as mean±SD. Statistical comparisons before and after maturation were determined using paired t test for paired data as appropriate.

RESULTS

Patient's Characteristics

The serum levels of IgG, IgA and IgM were lower than two standard deviations from the normal population in all patients (Table 1).

CVID patients had normal number of CD4+ T cells, 3 had increased numbers of CD8+ T cells, 3 had CD4/CD8 ratios less than 1.0 and all patients had normal CD19+ B cells. XLA patients had CD3 higher than normal range and CD19 lower than 1% (the hallmark of XLA patients).

Characteristics of Patients and Normal Serum Treated Monocyte-Derived DCs (Mo-DCs)

The freshly isolated monocyte from normal donors expressed high levels of CD14 and low levels of CD86, HLA-DR and CD11c. After 5 days culture of adherent monocytes in the presence of GM-CSF, IL-4 and samples' sera, monocytes acquired the phenotype of immature Mo-DCs; these cells characterized by decreased number of CD14 and increased densities of CD86, HLA-DR and CD11c.

The expression of CD86 and HLA-DR were elevated in 7-day culture mature DCs, but up regulation of co stimulatory markers was significantly higher in normal and XLA serum conditioned DCs than CVID serum conditioned DCs. The appearance of DCs was qualitatively similar for Mo-DCs cultured with GM-CSF and IL-4 in the presence of normal and CVID patient's serum. Mature DCs with many peripheral dendrites were seen at day 7.

Table 1. Immunological parameters of patients with common variable immunodeficiency and X-linked agammaglobulinemia.

Patients	Diagnosis	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD4/CD8 Ratio
1	CVID	80	17	8	70	38	31	9.9	1.22
2	CVID	233	4	4	86	41.56	45.7	6.37	0.9
3	CVID	225	70	50	63	29	23	10	1.26
4	CVID	170	0	0	79.4	40	45	10.7	0.89
5	CVID	140	13	14	77	31	25	12	1.24
6	CVID	469.8	36		69.6	29.1	38.99	7.8	0.74
7	CVID	114	52	30	66.38	35.23	31.29	24.9	1.12
8	CVID	76	0	11	81.08	44.14	31.9	9.78	1.38
9	CVID	103	28	30	65	31.7	34.2	31.4	0.93
10	CVID	160	50	68	81.08	44.14	31.98	9.7	1.38
11	XLA	0	0	0	93	42.84	45.97	0.006	0.93
12	XLA	90	15	27	89	62	23.4	0	2.65
13	XLA	200	6	20	91	50.9	33	0.8	1.54
14	XLA	30	0	0	92	16.3	73	0.58	0.22
15	XLA	90	0	0	91.4	60.2	17	1.1	3.54

Table 2. IL-18 production by mature DCs generated in the presence of sera from CVID, XLA and normal controls.

Samples	Diagnosis	Sex	Age (years)	IL-18 (ng/ml)
1	CVID	Male	26	4.680
2	CVID	Male	27	12.180
3	CVID	Male	12	3.166
4	CVID	Female	55	3.743
5	CVID	Male	8	6.000
6	CVID	Male	10	7.070
7	CVID	Female	11	7.430
8	CVID	Male	47	8.220
9	CVID	Male	14	7.170
10	CVID	Female	27	7.810
11	XLA	Male	11	6.680
12	XLA	Male	12	4.180
13	XLA	Male	20	8.060
14	XLA	Male	10	8.130
15	XLA	Male	5	8.360
16	Normal	Male	27	4.117
17	Normal	Female	30	2.126
18	Normal	Male	24	2.931
19	Normal	Male	27	4.060
20	Normal	Male	30	3.870
21	Normal	Male	40	3.900
22	Normal	Male	25	3.660
23	Normal	Male	30	3.700

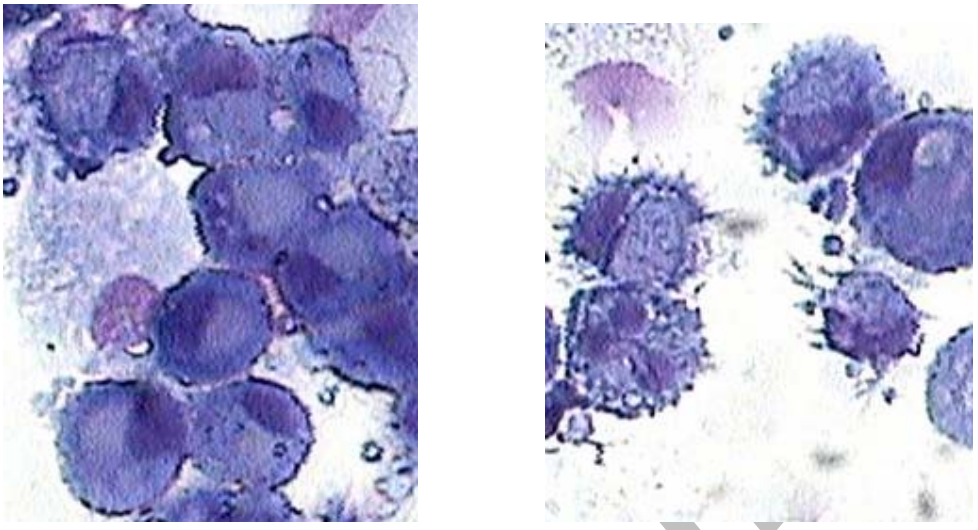
Production of IL-18 by DCs Generated in the Presence of Patients and Normal Sera

In order to have optimal conditions for cytokine production in mature DCs, CD40L (0.5-1 μg/ml) was used for 24 hours. IL-18 production by DCs generated in the presence of CVID patients' sera was 6.75±2.59 ng/ml, which was significantly higher than 3.55±0.68 ng/ml in normal sera conditioned DCs (P=0.003) (Table 2). The secretion of IL-18 from DCs generated in the presence of XLA patients' sera (7.08±1.75 ng/ml) was not different from the CVID values, while it was also significantly higher than normal values. (P=0.004) Figure 2.

DISCUSSION

The impaired DC-mediated T cell activation could be a consequence of the cytokine production pattern of DCs. According to plasticity hypothesis, different types of T cell–mediated immune responses can be induced by different types of DCs, which have different functions and cytokine patterns.[27] Thus, environmental conditions (cytokine milieu) may alter function of DCs to meet the needs of flexibility and plasticity.[28]

Although CVID is usually characterized by defective immunoglobulin production by B cells, a substantial proportion of CVID patients also seems to have some kinds of T cells defects, [4, 29] possibly due to failure of T cell to help B cells.



A B

Figure 1. Dendritic cells were generated from peripheral blood monocytes in the presence of GM-CSF, IL-4 and 10% of serum samples (patients and healthy donors). After 5 days immature DCs developed swollen processes (A) and changed to mature DCs after 2 additional days culture with TNF-α and MCM (B). The slides of DCs were prepared by Cytospine and stained with Wright-Giemsa.

In vivo evidence suggests that IL-18 shapes the development of adaptive immunity toward Th1 through modulation of plasmacytoid DC (DC2) function. The functional expression of IL-18R on pre-DC2 suggests an unexpected role for IL-18 in the recruitment of pre-DC2s to sites of inflammation. It is evidenced that DCs are able to secrete IL-18 that is able to induce IFN-γ production and Th1 differentiation in primed T cells.[20] Both IL-12 and IL-18 are produced by macrophages and DCs in response to microbial stimulation and act in a synergistic manner on T cells, polarizing them into Th1. Little is known about IL-18 production by DCs of XLA and CVID and its inducers. Herein we found that after CD40L stimulation, XLA and CVID serum treated DCs significantly up-regulated secretion of IL-18 as compared to healthy volunteers' sera treated DCs.

In vivo evidence suggests that IL-18 shapes the development of adaptive immunity toward Th1 through modulation of DC2 function.[30]

The factor leading to release of IL-18 has not been extensively studied. The functional expression of IL-18R on pre-DC suggests an unexpected role for IL-18 in the recruitment of pre-DCs to sites of inflammation.[30,31] There are some evidences showing that increased expression of IL-18R on a subset of naïve T cells has a profound role in induction of Th1 response in CVID patients.[31]

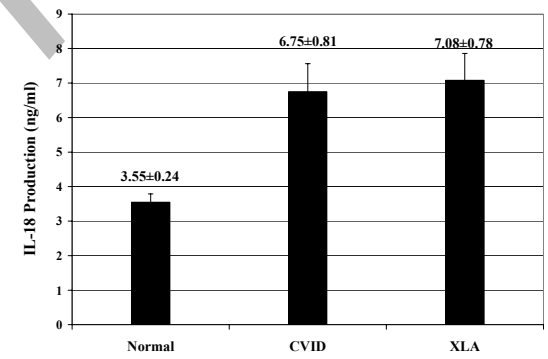


Figure 2. IL-18 production by mature DCs generated in the presence of CVID, XLA and normal sera. Mature DCs were stimulated with 0.5-1 μg/ml of CD40L and supernatant was collected for cytokine assay after 24 hours incubation. The results were shown as mean±SE.

Moreover as CVID patients are susceptible to recurrent infections, their serum may contain some kinds of lipopolysaccharides, which could stimulate DCs *in vitro* for high production of IL-18; however, further studies should be done to confirm the presence of lipopolysaccharides in the serum of the patients.

DCs and their function have an important role in the mechanism of the antibody deficiency in some CVID

patients.[32] Our results cumulatively indicate that some factors in CVID and XLA patients' sera can alter DC function to a defective phenotype, which may lead to defects in T lymphocyte proliferation and differentiation. Further studies could be conducted to shed light on the nature of these factors and define their primary or secondary role on DC function in primary antibody deficiencies.

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