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A novel IgA/Delta-like 4/Notch axis induces immunosuppressive activity in human dendritic cells☆

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ABSTRACT

We aimed to study whether IgA or IgG could modulate dendritic cells through the Notch pathway. Blood myeloid DC were isolated from controls or patients with allergic rhinitis (sensitized to house dust mite, *Dermatophagoides pteronyssinus*, Der p) and assayed for Notch ligand, Delta-Like 4 (DLL4) expression and co-cultured with allogeneic CD4 + T cells. An upregulation of DLL4 was observed in IgA-treated, but not IgG-treated DCs. In co-culture of DCs and T cells, pulsing DCs with IgA downregulated IL-13, IL-5 and IFN-γ responses, but upregulated IL-10. The suppressive effect of IgA was mediated by DLL4 not by IL-10. In contrast, IL-10 was required for the inhibition by IgG-DCs. Altogether, IgA imprints myeloid DCs with suppressive effects on CD4 + T cell responses to Th2 and Th1 antigens through activation of DLL4/Notch pathway, whereas IgG does not induce DLL4 expression, its inhibitory effects mainly relying on IL-10.

1. Introduction

Dendritic cells (DCs) recognize pathogens and allergens to induce adaptive immune responses. This role includes a complex spectrum of events, including antigen processing, cell maturation, migration and cross-talks through cytokines and cell-cell contacts with other immune cells. Current prevailing hypothesis is that asthma and allergy are associated with an aberrant immunity to environmental antigens, which includes $CD4^+$ Th2-biased responses to allergens and probably results from signals provided by mucosal epithelial and DCs [1]. Our previous study shows that DCs from allergic rhinitis (AR) and asthma patients display impaired responses to TLR activation for IL-12 (TLR4) and IFN-α (TLR9) production, as well as for induction of $CD4^+$ T cells producing IL-10 [2]. In addition, DCs from AR express lower ICOSL [3] and increased TSLPR [4], features which are further favoring Th2 responses.

Immunoglobulins are important host factors that may regulate DCs. IgE is the hallmark of allergy [5]. It mediates allergic reactions and meanwhile plays a role in immune regulation, ranging from allergic rhinitis and asthma to life-threatening anaphylactic shock [5]. IgE binds to the high-affinity receptor FcεRI which is expressed on basophils, mast cells, DCs, monocytes and eosinophils, and It also binds to the low-affinity receptor FcεRII (CD23) which is expressed on activated B cells, macrophages, eosinophils, T cells and structural cells [6]. Cross-linking of IgE-FcεRI complex on the mast cell and basophil surface leads to degranulation and synthesis of inflammatory mediators. $FcεRI^+$ DCs have been shown to be necessary and sufficient for the induction of Th2-driven allergic airway inflammation in response to inhaled house dust mite [7]. At one side, expression of FcεRI on DCs is important for igE-mediated antigen uptake and thus amplifying the late allergic response [8], but at the other side, IgE is rapidly endocytosed, transported to the lysosomes and degraded and may limit allergic responses within mucosal tissues [9,10]. IgG is a crucial immunoglobin to neutralize pathogens and activate the complement [11]. Intravenous IgGs are frequently used to treat autoimmune disorders [12]. Thus, IgG could modulate immune responses *via* their action on DCs [13] by signaling through Fcγ-receptors and the newly identified IgG-receptors DC-SIGN and dendritic cell immunoreceptor (DCIR) [14]. Similarly, IgA, which has a central role in host defense at mucosal surfaces [15], has a protective role against allergy and inflammatory diseases [16–18]. IgA may regulate DCs though FcαRI (CD89) to induce IL-10 expression [19] while inhibiting IL-12 production [20]. In allergy, both IgG and IgA antibody responses occur following allergen immunotherapy of pollen-induced rhinitis and asthma [21]. Thus, whereas IgG and IgA may inhibit cell activation *via* ITIM-coupled FcγRIIb [22] or monovalent ligation of FcαRI [23], respectively, a detailed and integrated view of the mechanisms by which IgA and IgG provide immunosuppressive signals to DCs is lacking.

Abbreviations: AR, allergic rhinitis; DC, dendritic cells; DLL4, delta like ligand 4; Der p, *Dermatophagoides pteronyssinus*; MD-DC, monocytes-derived dendritic cells; RT, room temperature.

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Notch represents a pleiotropic pathway regulating development and T cell growth. Mutations in this transcription factor can result in uncontrolled cell growth [24]. Studies have shown that DCs express Notch ligand delta-like 4 (Delta-4, DLL4) upon LPS-induced maturation [25–27], which promotes Th1 polarization [25,28,29] as well as Th17 induction upon DLL4 overexpression [30,31]. In particular, inflammatory DCs with high DLL4 expression induced significantly more IFN-γ- and IL-17-producing effector T cells [32], whilst in a GVHD model, inhibition of DLL4 inhibited *T*-cell production of IFN-γ [33]. Together these data indicate a role for DLL4 in Th1/17 commitment, whereas in the presence of exogenous recombinant DLL4, DC-driven IL-4 production by $CD4^+$ T cells is decreased [28]. Accordingly, *in vivo*, DLL4 blockade increased airway hyperresponsiveness and Th2 responses in a murine viral-induced asthma model [34]. Recent studies also showed that DLL4/Notch pathway is important for thymic DC-mediated Treg homeostasis, DLL4 blockade preventing the development of type 1 diabetes [35] and experimental encephalomyelitis [36] *via* Treg expansion. Collectively, these data indicate that DLL4 may tune immune responses by affecting the development and/or activation of several T cell subsets, typically promoting Th1/Th17 and inhibiting Th2 and Treg responses.

The aim of this study was to investigate whether IgA could regulate DCs through the DLL4/Notch pathway and, if so, its contribution to the regulation of $CD4^+$ T cell responses to Th1 and Th2 antigens and its comparison to IgG-mediated immunosuppression.

Table 1
Patient characteristics

	Allergic Rhinitis	Controls
N	20	23
Age (mean ± SD)	40 ± 13	48 ± 18
Sex	9M:11F	14M:9F
Concomitant asthma, n	8	0
Smoking, n	3	5
Topical steroids, n	10	0
Total IgE (U/ml), median (range)	172 (36 - 3,260)	Nd
Der p-IgE (U/ml), median (range)	10.3 (0.52 - 100)	Nd

Topical, intranasal (n = 10) +/- inhaled (n = 5), steroids were at low dose and withdrawn for at least 48hrs before blood sampling. Der p-IgE, *Dermatophagoides pteronyssinus*-specific IgE in serum.

2. Methods

2.1. Patients

Healthy non-atopic individuals and atopic patients with allergic rhinitis to *Dermatophagoides pteronyssinus* (Der p) were recruited (Table 1) for a leukapheresis to isolate blood DCs. The study was approved by the local Ethical Committees (Cliniques universitaires St-Luc, Brussels, Belgium) and was performed with the written informed consent from subjects.

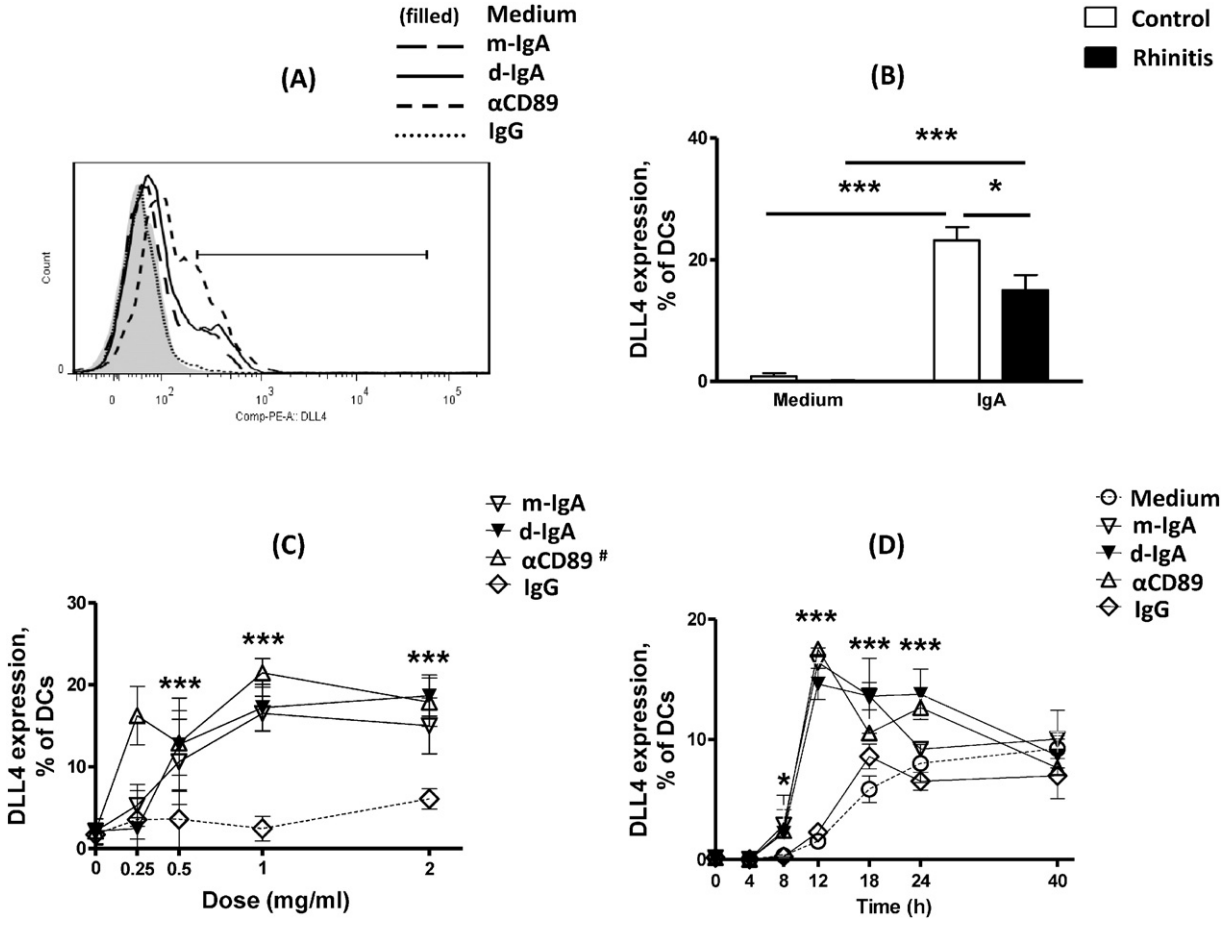

Fig. 1. IgA induces DLL4 expression in DCs. (A) DCs were cultured for 18 h with d-IgA, m-IgA, IgG (all 1 mg/ml), or anti-CD89 crosslinking (10 μg/ml) or medium, before staining and FACS analysis. Data represent means ± SEM (*$P < 0.05$, ***$P < 0.001$, IgA *vs* medium). (A) Representative histograms of DLL4 expression in DCs from a control donor; % of DLL4+ cells were calculated in the gated area after deduction of background (filled histogram). (B) DLL4 expression in IgA-DCs from controls ($n = 10$) and from patients with allergic rhinitis ($n = 12$). (C) Dose-dependent effect of IgA on DLL4 expression ($n = 5$ independent experiments; # concentration of anti-CD89 Ab is 2.5, 5, 10 and 20 μg/ml). (D) Kinetics of DLL4 induction ($n = 3$ independent experiments).