

ORIGINAL ARTICLE

B7-2/CD28 costimulatory pathway in children with atopic dermatitis and its connection with immunoglobulin E, intracellular interleukin-4 and interferon-gamma production by T cells during a 1-month follow-up

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Abstract

Background The best defined costimulators for activation of T lymphocytes are B7-1 and B7-2 on antigen presenting cells (APC) that bind to CD28 on T cells. Several studies showed that CD28 is critical for type 2 T helper cells (Th2) inflammation and depends mainly on the interaction of CD28 with B7-2. Some authors suggested a role for B7-2 B cells in immunoglobulin E (IgE) synthesis.

Objective We decided to study B7-2/CD28 interaction in atopic dermatitis (AD) and correlations with total and specific IgE, intracellular interleukin 4-(IL-4) and interferon-gamma (IFN- γ) production during a 1-month follow-up.

Methods We studied 24 AD children with allergy to cow's milk. Lymphocyte subsets (B7-2 on B cells, CD28⁺ on T cells, IL-4 and IFN- γ producing T helper cells), total and specific IgE, and IgG4 at days 1 and 30 were also studied. Scoring of atopic dermatitis (SCORAD) significantly decreased.

Results CD28⁺/CD3⁺/CD57 correlated with B7-2 B cells at days 1 and 30, with IL-4 and IFN- γ producing T helper cells at day 1 and with SCORAD at day 30. B7-2 B cells negatively correlated with IgE at day 30. Percentage of B7-2 B cells negatively correlated with total and specific IgE at day 30.

Conclusion Our results support the importance of CD28/B7 costimulation in AD children and the relation of CD28 with Th1 and Th2 cytokines. However, our results do not confirm the hypothesis about the preferential role of B7-2 in Th2 activation and IgE synthesis. It could raise a question about B7-2 blockade efficacy in AD children. Further investigations on B7 family members and their functions could help to distinguish target for more clinically efficient B7/CD28 blockage in AD.

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Keywords

atopic dermatitis, CD28, CD86, B lymphocyte, immunoglobulin E, T lymphocyte

Conflicts of interest

None declared.

Introduction

Activated T and B lymphocytes play a crucial role in the pathogenesis of atopic dermatitis (AD).¹ Several costimulatory pathways for T- and B-cell activation are currently identified. The best defined costimulators for T lymphocytes are B7-1 (CD80) and B7-2 (CD86) molecules on antigen-presenting cells that bind to members of the CD28 family receptors on T cells. The first of these receptors for B7-1 and B7-2 to be discovered was the CD28 molecule. Binding of B7 molecules on antigen-presenting cells to CD28 delivers signals to the T cells that induce the expression of anti-apoptotic proteins, stimulate production cytokines, and promote T-cell proliferation and differentiation. CD28 is the principal

receptor for delivering second signals for T-cell activation. The biochemical mechanism by which CD28/B7 interaction promotes T-cell activation is incompletely understood. A second receptor for B7 molecules was discovered later and called CTLA-4 (CD152). CTLA-4 is structurally homologous to CD28 and is a receptor for both B7-1 and B7-2. Its function is to inhibit T-cell activation by counteracting signals delivered by CD28. In B cells, engagement of the antigen receptor stimulates B7 expression, so that B cells that encounter antigen are able to activate and be helped by CD4 T cells. On the basis of many experimental studies antagonists against B7 molecules are in clinical trials to prevent the rejection of organ allografts and other pathologic immune responses.²

It has been demonstrated in animal models that the Th2 response requires continuing costimulation via CD28, and treatment with CD80 and CD86 antibodies inhibits immunoglobulin E (IgE) production in mice.^{3,4} CD28 activation is important in Th2-mediated inflammation.⁵ CD28 signalling regulates the threshold of T-cell receptor (TCR) engagements needed for effective T-cell activation.⁶ The importance of CD28/B7 pathway in the *in vivo* immune response to allergen was described in studies examining the effects of B7 blockade with CTLA-4-Ig, a soluble fusion protein inhibitor of B7-1 and B7-2.⁷⁻⁹ Several studies showed that CD28 stimulation is critical for Th2 differentiation, although contradictory findings have been reported.¹⁰ Increasing CD28 ligation resulted in increased production of interleukin-4 (IL-4) in transgenic mice expressing a TCR specific for ovalbumin.¹¹ Human naïve cord blood CD4⁺ T cells are not primed to produce Th2 cytokines in the absence of CD28 costimulation and consequently default to the Th1 subset independent of the presence of exogenous cytokines.¹² Stimulation via CD28 elicits Th2 differentiation of naïve CD4⁺ T cells particularly efficiently in the context of low TCR signalling.¹³ CD28-deficient mice have preserved Th1-mediated cellular immunity accompanied by low basal serum levels of the Th2-dependent IgG1 isotype and high levels of the Th2-dependent IgG2a isotype.¹⁴ In another model of transgenic mice expressing a TCR specific for ovalbumin, CD28-deficient mice displayed reduced IL-4 and proliferative responses to ovalbumin, while interferon-gamma (IFN- γ) production was normal.¹⁵ CD28 cytotoxic T lymphocytes are good producers of IFN- γ while IL-4 prevents loss of CD28 expression on these T cells.^{16,17} Studies in mice have suggested that the generation of Th2 cells depends mainly on the interaction of CD28 with B7-2. In the murine experimental allergic encephalomyelitis model, treatment with anti-B7-1 monoclonal antibody leads to Th2 responses while anti-B7-2 monoclonal antibody has the opposite effect.¹⁸ *In vitro* studies of human T cells reported that B7-2 transfectants skewed responses towards the production of Th1-type cytokines. It was shown that B7-2 provided an initial signal to induce naïve T cells to become IL-4 producers.¹⁹ Other studies found that both B7-1 and B7-2 were able to costimulate IL-4 and IFN- γ production from murine T cells.^{20,21} In the study by Jirapongsananuruk *et al.*,²² it was shown that expression of B7-2 on B cells of adult AD patients was significantly higher than non-atopic controls and psoriatic patients. In contrast, the authors did not find significant difference in B7-1 expression among three mentioned subject groups. Total serum IgE from adult AD patients and normal subjects in that study correlated significantly with percentage levels of B7-2 expression on B cells, suggesting a role for B7-2⁺ B cells in IgE synthesis.

Zdolsek and Jenmalm in their study did not find any differences in CD28 expression within CD4⁺ T lymphocyte subsets between atopic and non-atopic children.²³

We decided to study B7-2/CD28 interaction in children with AD and its connection with intracellular IL-4 (Th2) and IFN- γ

(Th1) production by T cells, total serum IgE levels and specific IgE to the most spread food allergen in this age group – cow's milk²⁴ – during a 1-month follow-up period. Because of the reports that CD57⁺/CD28⁻ T cells display a typical Th1-type cytokine secretion profile upon anti-CD3 stimulation, with a markedly higher secretion of the Th1-type cytokines IL-2, IFN- γ , and TNF- α than their CD57⁻/CD28⁺ counterparts,²⁵ we decided to study CD28 expression on CD57⁻ T cells as more relevant to Th2 process.

Materials and methods

Subjects

We studied 24 AD children (4 years or younger) with allergy to cow's milk confirmed by specific IgE and/or specific IgG and anamnestic data. The children's mean age was 17.29 ± 11.20 months. The diagnosis of AD was made using the Hanifin and Rajka criteria.²⁶ Disease severity was estimated by the same dermatologist using SCORAD at days 1 and 30.²⁷ At day 30, significant decrease of SCORAD from 30.88 ± 15.59 (day 1) to 18.38 ± 9.79 ($P < 0.01$) was reported. During the follow-up period, all children used Trixera (Laboratoires Dermatologiques Avene, Paris, France) emollient cream and Trixera emollient preparation for bath as moisturizers. On the basis of recent information that *Lactobacillus* could suppress B7-2 gene expressions in mice²⁸ and about the clinical benefits of probiotics, especially *Lactobacillus rhamnosus*, in children with AD,²⁹ we decided to add probiotic preparation to the treatment. Probiotic preparation Lacidofil (Institut Rosell Inc., Montreal, Quebec, Canada) (containing 95% *L. rhamnosus* Rosell-11 and 5% *Lactobacillus acidophilus* Rosell-52), one capsule per day, was included to complex treatment. Ethical permission for the study was granted by the local ethics research committee. The parents of the children gave written informed consent.

Flow cytometry of lymphocyte subsets

Lymphocyte subsets were identified by three-color flow cytometry. Erythrocyte-lysing whole blood method of lymphocyte staining by FITC-, PE- or PE-Cy5-conjugated monoclonal antibodies to the following human lymphocyte subsets: CD3/CD28/CD57- and CD19/CD86 (Becton Dickinson, San Jose, CA, USA) was used. Stained samples were analysed by FACScan flow cytometer using FACScan Research & Lysis software (Becton Dickinson). For defining lymphocyte gate, whole blood samples were incubated with LeucoGATE (anti-CD45-FITC and anti-CD14-PE monoclonal antibodies, Becton Dickinson) to permit discrimination of lymphocytes from granulocytes, monocytes, debris, and unlysed or nucleated red blood cells. As background control, whole blood samples were incubated with SimulTEST control (IgG1-FITC + IgG2-PE, Becton Dickinson).

Detection of intracellular cytokines in activated lymphocytes

For the flow cytometric analysis of peripheral blood mononuclear cells, samples were incubated for 4 h with ionomycin, brefeldin A

and phorbol 12-myristate 13-acetate (all from Sigma, St Louis, MO, USA), then the following monoclonal antibodies were used to detect the surface and intracellular antigens: IFN- γ /IL-4 (Becton Dickinson). Three-colour flow cytometric analysis was carried out using FACScan (Becton Dickinson). A total of 1×10^4 cells were estimated. Th1 cells were defined as CD4⁺ lymphocytes with intracellular IFN- γ but without IL-4. Th2 cells were detected as CD4⁺ lymphocytes with intracellular IL-4 but without intracellular IFN- γ .

Total IgE, specific IgE and specific IgG4 to cow's milk in serum of AD patients were studied by ELISA using commercial kit by Dr Fooke Laboratories GmbH (Mainstraße, Germany). All studies were done at days 1 and 30.

Statistical methods

Data were presented as mean percentage $\pm$ standard deviation. Wilcoxon matched pairs test and Spearman non-parametric correlation (Spearman r) were used. The results were considered significant if $P < 0.05$.

Results

Counts of all lymphocyte subsets that have been studied did not achieved statistically significant difference (Table 1). Total and specific IgE to cow's milk levels in serum of children with AD also did not differ significantly before and after the treatment (218.57 ± 629.65 and 198.65 ± 619.06 IU/mL for total IgE; 8.48 ± 15.17 and 6.57 ± 26.17 IU/mL for specific IgE). Levels of specific IgG4 to cow's milk significantly increased from 129.97 ± 127.44 μ g/mL at day 1 to 146.59 ± 130.48 μ g/mL at day 30 ($P < 0.05$).

Only significant correlations are reported below. High levels of correlation between CD28⁺/CD3⁺/CD57⁻ T cells and B7-2 on CD19⁺ B cells at day 1 ($r = 0.59$, $P < 0.01$) and day 30 ($r = 0.58$, $P < 0.01$) were noted. CD28⁺/CD3⁺/CD57⁻ T cells correlated with IL-4 producing T helper cells ($r = 0.45$, $P < 0.05$) as well as with IFN- γ producing T helper cells ($r = 0.39$, $P < 0.05$) at day 1 and with SCORAD ($r = 0.78$, $P < 0.01$) at day 30. B7-2 B cells negatively correlated with total IgE levels ($r = -0.41$, $P < 0.05$) at day 30. Percentage of B7-2 B cells negatively correlated with both total IgE levels ($r = -0.49$, $P < 0.05$) and specific IgE ($r = -0.68$, $P < 0.01$) at day 30. IFN- γ producing T helper cells correlated with total IgE ($r = 0.50$, $P < 0.05$) and specific IgG4 ($r = 0.48$, $P < 0.05$) levels at day 1.

Discussion

Our results support the importance of CD28/B7 costimulatory pathway in children with AD on different stages of inflammation.

Despite of the fact of strong correlation between B7-2 B cells and CD28 T cells quite a small portion of B cells expressed B7-2 molecule meanwhile majority of T cells were CD28 positive. The majority of CD28 on T cells should probably interact with B7 molecules on antigen presenting cells other than B cells (i.e. Langerhans cells). Correlation of B7-2 B cells with CD28 T cells after significant decrease of clinical signs and strong correlation of CD28 expression with SCORAD on day 30 could support the hypothesis about the important role of CD28/B7 interaction in mechanisms of chronic stimulation in AD patients. Despite the contradictory findings about the role of CD28 in Th1 and Th2 cytokine production,^{10–12,15} we reported correlation of CD28 with both IFN- γ (Th1) and IL-4 (Th2) production by T helper cells at day 1 and absence of these correlations at day 30. We also found extremely poor correlation between IgE and IL-4 production as previously shown by van der Pouw Kraan *et al.*³⁰ and surprisingly found correlation of IFN- γ producing T helper cells with total serum IgE in children with AD.

Our results could not confirm the hypothesis about the preferential role of B7-2 in Th2 activation and IgE synthesis.¹⁹ We also failed to find correlation of serum IgE with B7-2 expression reported by Jirapongsananuruk *et al.*²² at day 1 and, moreover, reported negative correlation of B7-2 with total and specific serum IgE of AD children at day 30. Suppressive effect of B7-2 antibodies on IgE production in mice was previously reported by Chapoval and David.⁴ Our findings could raise a question about B7-2 blockade efficacy in particular in children with IgE-dependent AD.

The role of IgG4 in food allergy is highly controversial.³¹ According to the European Academy of Allergology and Clinical Immunology (EAACI) Task Force Report, IgG4 should not be considered as a factor that induces hypersensitivity, but rather as an indicator for immunological tolerance, linked to the activity of regulatory T cells.³² Food-sensitized infants with high levels of IgG4 to food allergens are more likely to consume these foods at the age of 4 years than infants with low IgG4 levels.³³ It was also shown that maintenance of tolerance to cow's milk in atopic children and adults without cow's milk allergy (CMA) is associated with elevated levels of specific IgG4, in combination with low specific IgE.³⁴ In this context, we can speculate that significant increase of specific IgG4 levels associated with decreased SCORAD in our study could be consider as a shift to tolerance.

Zdolsek and Jenmalm did not find any differences in CD28 expression within CD4⁺ T lymphocyte subsets between atopic and non-atopic children.²³ Choi *et al.* found significantly up-regulated expression of another receptor for B7 (CTLA-4) in peripheral

Table 1 Levels of lymphocyte subsets in peripheral blood of children with atopic dermatitis at days 1 and 30 ($n = 24$)

	Day 1	Day 30
B7-2 on CD19 ⁺ B cells	$32.62 \pm 21.82 \times 10^6$ cells/L	$42.23 \pm 30.05 \times 10^6$ cells/L
CD28 ⁺ on CD3/CD57 ⁻ T cells	$3543.44 \pm 2111.60 \times 10^6$ cells/L	$3156.92 \pm 1079.10 \times 10^6$ cells/L
IFN- γ producing T helper cells	$101.80 \pm 99.30 \times 10^6$ cells/L	$77.90 \pm 30.05 \times 10^6$ cells/L
IL-4 producing T helper cells	$43.50 \pm 24.74 \times 10^6$ cells/L	$48.30 \pm 43.24 \times 10^6$ cells/L

blood T cells of patients with AD, compared with normal controls.³⁵ CTLA-4 is homologous to CD28 and inhibits T-cell activation by counteracting signals delivered by CD28.² CD28 expression in AD children is not upregulated²³ but well correlated with clinical severity of AD after significant decrease of clinical manifestation according to our results. Expression of CTLA-4 reported to be increased in peripheral T cells of AD patients³⁵. CTLA-4 has blocking function on T cell activation² that play crucial role in the pathogenesis of AD. Mentioned above facts make it possible to speculate that CD28 may be involved in mechanisms that support chronic pathologic process in AD even when clinical signs are diminished or almost absent and even increased level of CTLA-4 is not enough to suppress this process. In this case attempts to block B7-1 and B7-2 with CTLA⁷⁻⁹ could be unsuccessful. Further investigations on B7 family members molecules and its functions could help to distinguish target for more clinically efficient B7/CD28 blockage in AD patients.

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