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PEANUT-SPECIFIC RESPONSES IN YOUNG CHILDREN WITH ATOPIC DERMATITIS

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INTRODUCTION

In young children atopic dermatitis (AD) is one of the first clinical signs of the presence of atopy, while asthma and certainly rhinitis is associated with later ages. AD is an intensely pruritic inflammatory skin disorder. Food allergens besides genetic determinants are considered to play an important role in the pathogenesis of AD. Approximately 60% of children with severe AD have allergic reactions to food constituents: mostly cow's milk, egg, peanut and soy. Peanut allergy starts often early in life, is usually severe and does not resolve. Seven percent of allergic reactions caused by peanuts are anaphylactic reactions.
In children with AD the immuno pathology is based on an abnormal cytokine regulation, specifically by helper (CD4+) T cells. Helper T (Th) cell subsets are considered the major source of those cytokines that are implicated in the expression of atopic symptoms. Th cells can be divided in Th1 and Th2 cells based on a mutually exclusive cytokine production profile. Activated Th2 cells secrete a variety of cytokines, including IL-4, IL-5, IL-10 and IL-13, which play a role in the induction of IgE formation and skin infiltration by eosinophils and T cells. Furthermore, Th2 cytokines can down-regulate Th1 activity, which consist mainly of Interferon-γ (IFN-γ) production.

METHODS

Identification of proliferating T cell subsets is permitted by flow cytometric analysis of cells, double stained for CD4 or CD8 surface markers and the Ki67-antigen. Ki67-antigen is a nuclear antigen that is expressed in actively cycling cells, but not in resting G_0 cells. Ki67-antigen levels increase during the S phase with a maximum in the G_2/M phase of the cell cyclus.
The proliferative responses of the T cell subsets were analyzed by [^{3}H]-thymidine incorporation and by the expression of the intracellular Ki67-antigen after peanut-extract specific stimulation of peripheral blood mononuclear cells (PBMC) of three different patient groups. We studied children with AD with a peanut-allergy (AD^+PA^+, n=24), children with AD without a peanut-allergy (AD^+PA^-, n=11) and healthy children (HC, n=21).

RESULTS

Peanut-extract induced proliferative responses of PBMC from AD^+PA^+ were significantly increased as compared to the two control groups AD^+PA^- and HC (Table 1). Ki67-antigen double staining revealed that 80-100% of the proliferating cells were $CD4^+$.

	atopic dermatitis		healthy children
	PA^+ (n=21)	PA^- (n=11)	(n=21)
Peanut-extract			
Baseline cpm	1721.6±1683.1	2840.7±2397.7	1472.8±1097.3
Total cpm	9283.7±8753.8	7040.1±7376.6	2283.6±2045.1
SI (range)	7.3 (1.6-21.0)	2.7*(1.0-5.9)	1.3*(1.0-3.2)

Table 1. *Proliferative responses of PBMC from children with atopic dermatitis with and without peanut-allergy and from healthy children*
PA^+= peanut allergic AD children; PA^-= AD children without peanut-allergy.
*PBMC were incubated for 7 days with peanut-extract (500 µg/ml) and then assessed for proliferation by [^{3}H] Thymidine incorporation. Results are expressed as * mean cpm ± SD or median SI (range).*
** Indicates p<0.05*

Next, we studied in these children relationships between proliferative responses, cytokine gene expression and production. Cytokine mRNA (IFN-γ, IL-4) was detected by a semi-quantitative RT-PCR method and expressed as scan values and cytokine production was measured by ELISA. The proliferative responses in AD^+PA^+ children correlated significantly with an increase in IL-4 mRNA expression after peanut-extract specific stimulation (Figure 1).

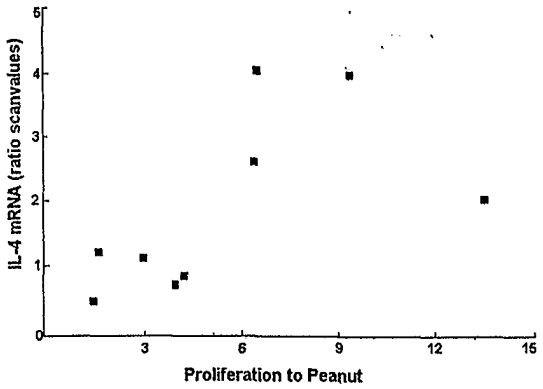

Figure 1: *Correlation between the proliferative responses to peanut (expressed as SI) and the ratio of IL-4 mRNA (expressed as ratio of scan value after peanut-extract specific stimulation over scan value of no addition) with a correlation coefficient of r_S=0.79 and p=0.019.*

CONCLUSIONS

We showed that after peanut-extract specific stimulation PBMC of AD children with peanut allergy had an increased proliferative response of predominantly CD4+ (helper) T cells. The correlation between IL-4 mRNA expression (which is a Th2 cytokine) and the proliferative response to peanut-extract specific stimulation suggest that the PBMC fraction of AD^+PA^+ children contains increased frequencies of peanut-specific T helper-2 cells.