

969 Limitations of the Physical Activities in Asthmatic Children and Teenagers

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The asthma can be direct or indirect cause of physical limitations in asthmatic patients affecting their quality of life. The objective was to identify the facts that influence and limits the physical activities in asthmatic children and teenagers. This was a descriptive and observational study in 83 asthmatic children and teenagers, between 6 to 18 years, who attended a summer camp for asthmatics patients. They answer a questionnaire to investigate the kind of physical activities that they regularly made and to identify the facts, that limit physical activities in each of these patients. While in the camp we observed the performance of the different physical activities they participated and identified those cases that they have to stop exercising because of an asthma crisis. For the statistical analysis we used central and percentage tendency. 60 patients (72.3%) practiced regularly some kind of sport being the most frequent soccer (36.7%), swimming (20.1%), athletics (8.4%) and basketball (8.4%). Of the 23 children and teenagers that didn't practice regularly any sport 19 of them were between 5 to 10 years. In the first group, 28 (46.7%) said that they have to stop exercising because they starting an asthma crisis. In the camp, 8 children (9.7%) had to stop their physical activities because an asthma crisis. 47 of them (56.6%) knew the kind of physical activity they could participate and the correct way to perform it; this was the result of an educational process. In conclusion: in our community the development of the asthma crisis and the lack of adequate physical education are the facts that influence the limitations of exercise in asthmatic children and teenagers.

970 Phenotype of Allergen-Specific T-Cells Derived From the Peripheral Blood of Peanut-Allergic Children

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Allergic immune responses are described by a Th2 cytokine profile. This is supported by work with allergen-specific T cell lines or clones requiring repeated cycles of stimulation which can result in altered cell surface phenotype and cytokine production. We have established a rapid technique for the identification and characterisation of allergen-specific T cells by FACS. Peripheral blood mononuclear cells (PBMC) from children with peanut allergy (PA) (n=5) were stimulated with peanut extract, ovalbumin, β -lactoglobulin, PPD, tetanus toxoid (TT). Antigen-specific cells were identified by FACS on the basis of their differential staining with a cellular fluorochrome (5,6-carboxyfluorescein succinimidyl diacetate ester) that had been used to label them at the start of the culture. We checked the allergen specificity of the gated population by cell sorting and subsequent cloning, showing that more than 90% of the gated population was antigen-specific. Cultured PBMC were stained for cell surface markers (CD3, CD4, CLA, β 7 integrin) while cytokine production (IFN γ , IL-4, IL-5, IL-13) was assessed using intracellular staining. More than 95% of cells that proliferate to peanut or control antigens in vitro are CD3+ CD4+. The percentage of IFN γ -producing peanut-specific T cells in PA children is lower than the percentage of IFN γ -producing T-cells specific for other antigens. Conversely, the percentages of IL-4, IL-5 and IL-13-producing cells are higher among peanut-specific T cells than among lymphocytes recognising other food antigens. Thus, we have used a novel technique to isolate and characterise antigen and allergen-specific T cell populations by FACS. We show that peanut-specific T cell responses in children with PA show a Th2 profile

similar to TT in contrast with the Th1 cytokine profile of the cell populations that respond to control food antigens and PPD. The funding for this study has been provided by the UK Food Standards Agency.

Percentage of cytokine-producing cells in allergen-specific cell populations

	IFN γ	IL-4	IL-5	IL-13
no antigen	31.9	3.8	1.1	4.1
peanut	40	8.4	2.2	15.5
lactoglobulin	77.2	5.3	1.3	2.6
ovalbumin	77.9	4.7	0.3	2.7
PPD	82.1	1.2	0.4	1.8
TT	52.5	12.6	1.2	10.9

971 Comparable T-Cell Activation Induced by Natural and Recombinant Bet v 1

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BACKGROUND: Specific allergy vaccination (SAV) was introduced in 1911. Since then the success rates of the treatment have increased due to the improved quality of the vaccines, better administration protocols and diagnostics. The next generation allergy vaccines have been suggested to be recombinant major allergens. Since naturally occurring major allergens are composed of many isoforms, it is necessary to investigate if all allergic patients will benefit from treatment with a single recombinant isoform.

AIM: In the present study we have tested if a single recombinant Bet v 1 isoform can replace the many isoforms included in natural Bet v 1 extracts for T-cell stimulation in allergic patients.

METHODS: Naturally occurring isoforms of Bet v 1 (nBet v 1, mixture of isoforms) were purified from birch pollen extract (Bet v). In addition a single Bet v 1 isoform was cloned and purified (rBet v 1). PBMC from birch allergic patients were stimulated with culture medium, Bet v, nBet v 1 and rBet v 1. Samples were tested for proliferation and cytokine production by CBA assay (IFN-gamma, IL-10, IL-5 and IL-4). From the same patients, T-cell lines were established by stimulation with nBet v 1. After 2 rounds of specific stimulation the T-cell lines were tested for reactivity towards Bet v, nBet v 1 and rBet v 1 the output being proliferation and cytokine production.

RESULTS: Ten PBMC cultures showed specific stimulation to nBet v 1. Seven PBMC cultures proliferated equally well to both rBet v 1 and nBet v 1, the remaining 3 PBMC cultures responded with a weaker signal to rBet v 1 compared to nBet v 1. Cytokine production was dominated by IFN-gamma production and increased proportionally with PBMC proliferation. In addition, eight T-cell lines were established and all except one proliferated equally well to both nBet v 1 and rBet v 1. Four T-cell lines showed a Th0 like cytokine pattern, three showed a Th1 pattern and one showed a Th2 pattern determined on the basis of the relative IFN-gamma and IL-5 production. The balance between IFN-gamma and IL-5 was not affected by the different stimulants.

CONCLUSION: All PBMC cultures and 7/8 T-cell lines that showed specific stimulation to nBet v 1 also responded to rBet v 1. These data suggests that for T-cell stimulation a single isoform of Bet v 1 can substitute for the mixture of individual isoforms found in the natural allergen preparations. Thus, vaccines based on recombinant allergens will address the existing Bet v 1 specific T-cell population.

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