

2. Stenton SC, Dennis JH, Hendrick DJ. Occupational asthma due to cef-tazidime. *Eur Respir J* 1995;8:1421-3.
3. Rose BG, Kemps-Holtzaple C, Stanker LH. Competitive indirect ELISA for ceftiofur sodium and the effect of different immunizing and coating antigen conjugates. *Bioconjugate Chem* 1995;6:529-35.
4. Wass U, Belin L. Immunological specificity of isocyanate induced IgE antibodies in serum from 10 sensitized workers. *J Allergy Clin Immunol* 1989;83:126-35.

doi:10.1067/mai.2003.1525

No difference in type 1 T-cell immune responses to human cytomegalovirus antigens between atopic children and nonatopic children

To the Editor:

The relationship between atopic phenotypes and T-cell function is not fully understood. The classic atopic manifestations, such as atopic dermatitis, bronchial asthma, and seasonal allergic rhinoconjunctivitis, are characterized by an increased IgE synthesis and a T_H2 -dominated immune response.¹

Viral infections are known to have a strong influence on the human immune system. Infections with human cytomegalovirus (HCMV) have a high prevalence and are lifelong. The infection is greatly dependent on type-1 cytokine secreting and cytotoxic T cells. Almost all HCMV-seropositive individuals express a type 1 T-cell response to HCMV.²

The structural phosphoprotein UL83 (pp65) and the nonstructural protein IE-1 are major targets of the $CD8^+$ T-cell response³; they were therefore selected for the present study. HCMV-pp65/IE-1-specific $CD8^+$ T cells account for more than 1% of our peripheral blood T cells.⁴ Specific stimulation of these $CD8^+$ T-cell subsets might therefore reflect $CD8^+$ T-cell reactivity.

Type 1 $CD4^+$ or $CD8^+$ cells produce key cytokines, such as IFN- γ , TNF- α , and GM-CSF, whereas type 2 T cells are characterized predominantly by secretion of IL-4, IL-5, and IL-13. Whereas type-2 T cells promote IgE production and eosinophilia, type 1 T cells induce cytotoxic and delayed-type hypersensitivity responses, stimulate secretion of complement-fixing antibodies (IgG1,3), and suppress the T_H2 function. Cytotoxic T lymphocytes eliminate virus-infected cells. Type 1 cytokines—in particular, IFN- γ —inhibit viral promoter activity and replication. A strong type 1 T-cell response suppresses type 2 responses and protects against atopic disease, at least in atopic dermatitis.⁵

More than 60% of adults in the industrial nations are HCMV-seropositive. Through use of highly sensitive PCR techniques, HCMV DNA is also detectable in at least 50% of seronegative blood donors,⁶ suggesting a high prevalence of HCMV exposure. The HCMV genome contains more than 200 open reading frames, possibly coding for as many proteins. An infection with HCMV leads to a strong T_H1 -type cytokine secretion pattern.

We evaluated whether atopic subjects characterized by a T_H2 -cytokine secretion pattern respond differently to T_H1 -inducing viral antigens in vitro. We used flow cytometry to analyze the HCMV-specific T-cell responses of children with and children without atopic disease.

Twenty-four atopic children were selected on the basis of a clinical diagnosis of asthma, allergic rhinoconjunctivitis and/or atopic dermatitis plus allergic sensitization (atopic dermatitis, $n = 9/24$; bronchial asthma, $n = 7/24$; allergic rhinoconjunctivitis, $n = 9/24$; allergic sensitization, $n = 24/24$). The mean age was 11.8 years (range, 2-17 years); 71% of the subjects were boys and 29% were girls. Allergic sensitization was defined as sensitization to at least one of the common aeroallergens or food allergens. Total and specific serum IgE levels were measured with the in vitro immunoassay CAP system (Pharmacia Diagnostics, Freiburg, Germany). Specific sensitization was defined as ≥ 0.35 kU/L.

We compared these individuals with 76 children who did not have any atopic history or clinical signs of atopy (without asthma, allergic rhinoconjunctivitis, atopic dermatitis, and sensitization). The children were matched for age and sex. The mean age in the control group was 10.3 years (range, 1-17 years); 54% were boys and 46% were girls. Children taking immunosuppressive medications, such as oral glucocorticosteroids, were not included in the study.

IFN- γ -positive T cells were identified by flow cytometry (FACS Calibur, Becton, Dickinson and Company, San Jose, Calif) after stimulation of heparinized blood samples with inactivated HCMV lysate and pp65 and IE-1 protein-spanning peptide pools. After 1 hour of stimulation at 37°C, brefeldin A was added (2.5 μ L/mL); 16 hours later, the cells were collected. The erythrocytes were lysed by BD Lysing solution, and the cell samples were stained for CD4, CD8, CD69, and CD3 surface antigens. PMA and Ionomycin were applied as positive controls. Negative controls and isotype controls were made to detect and eliminate potential unspecific reactivity.

A detectable T_H1 immune response based on IFN- γ -positive T cells was defined as a gated event result of $\geq 0.20\%$. In addition, the humoral immune response was analyzed by measurement of HCMV-specific IgG (Immulate, DPC-Biermann, Bad Nauheim, Germany).

Statistical significance was calculated by means of χ^2 and Yates tests through use of computer software (SPSS version 8.0); a P value of $< .05$ was considered statistically significant.

The overall prevalence of HCMV seropositivity in the study population of 100 children was 40%; 60% of all children were HCMV-seronegative. A type 1 immune response with an increase in IFN- γ -positive T cells after stimulation with at least one of the HCMV stimuli was measured in 50% (20/40) of the HCMV-seropositive children and in 6.7% (4/60) of the HCMV-seronegative children. Of the total study population of 100 children, a T_H1 immune response was detected in 31.3% after antigen stimulation ($CD4^+$ only), in 19% after pp65 stimulation ($CD4^+/CD8^+$), and in 29% after IE-1 stimulation ($CD4^+/CD8^+$).

The prevalence of HCMV seropositivity in the atopic children was 50%; this compared with 37% in the nonatopic children.

Comparing atopic children versus nonatopic children with respect to T_H1 reactivity, we found a type 1 immune

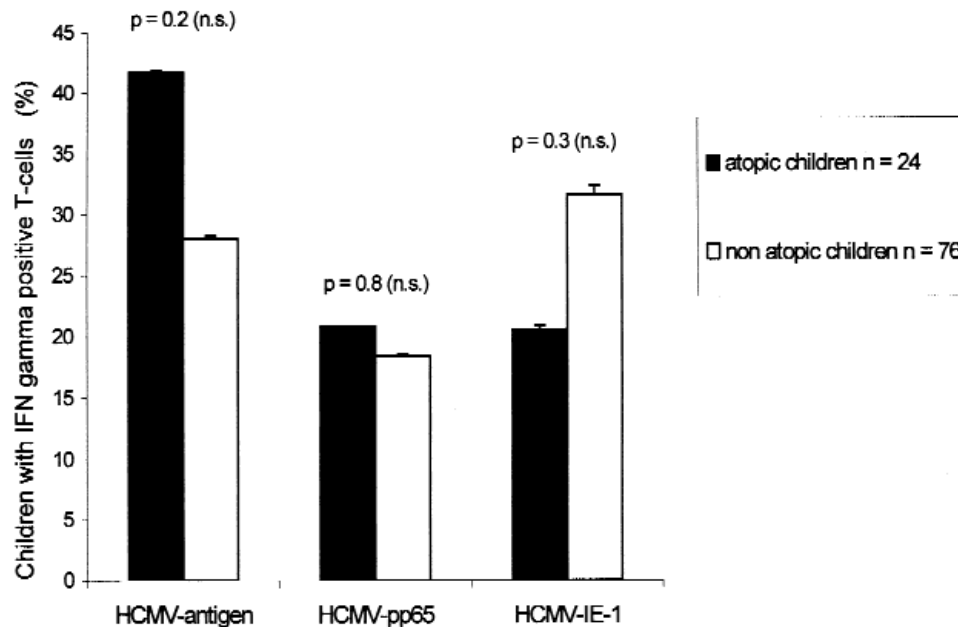


FIG 1. Quantitative differences in IFN- γ -positive T-cell counts between atopic children and nonatopic children after stimulation with HCMV antigens. IFN- γ -positive T-cell results were defined as gated events $\geq 0.20\%$. n.s., Statistically not significant.

response with HCMV antigen in 41.7% of those who were atopic and in 28% of those who were nonatopic; after stimulation with HCMV peptide-mix pp65, the figures were 20.8% versus 18.4%, and after stimulation with HCMV peptide-mix IE-1, the figures were 20.8% versus 31.6%. Negative controls never showed IFN- γ -positive cells $\geq 0.20\%$. The atopic children and the nonatopic children were not statistically different with respect to the percentage of IFN- γ -producing T cells after HCMV stimulation (Fig 1).

Analysis of the memory/effector T-cell reactivity to HCMV antigens in the atopic children and the nonatopic children after in vitro stimulation with cytomegalovirus did not reveal significant differences. There was no statistically significant difference between children with atopic disorders and children without atopic disorders regarding the frequency of IFN- γ -positive T-cells (neither CD8⁺ nor CD4⁺) after stimulation with HCMV antigens (total virus lysate, pp65, and IE-1 peptide pools).

Half of the seropositive children showed a type 1 response to HCMV. The higher incidence of nonresponders among the seropositive children⁷ in comparison with adults might result from lower frequencies of memory/effector T cells due to less-frequent HCMV reactivation. The difference might also be related to the method used. To decrease the amount of blood extracted, we used a whole-blood method that is slightly less sensitive than the stimulation of isolated PBMCs. However, the nonresponders cannot influence the overall result. The reaction of some seronegative children to HCMV is not surprising; similar observations have been made in adults.

Using new PCR methods, Roback et al⁶ could establish exposure to the virus in 50% of seronegative probands. The differences regarding the pp65 response versus the

IE-1 response reflect the variance of HLA types.³

Several studies indicate a protective effect of viral infection in early childhood with regard to atopy.⁸ However, our own observations could not demonstrate a protective effect for HCMV exposure.⁹ In the present study, the groups of atopic children and nonatopic children did not differ with respect to specific serum IgG to HCMV. In conclusion, there was no significant difference in anti-HCMV T-cell reactivity between children with an atopic phenotype and children without an atopic phenotype. Our results indicate that atopic disease cannot disturb a protective type 1 cytokine response to HCMV antigens.

Nora Laske, MD^{a,b}

Florian Kern, MD^b

Renate Nickel, MD^a

Ulrich Wahn, MD^a

Hans-Dieter Volk, MD^b

^aDepartment of Paediatric Pneumology and Immunology

^bInstitute of Medical Immunology

Charité, Humboldt University

Berlin

Germany

REFERENCES

1. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196-200.
2. Kern F, Bunde T, Faulhaber N, Kiecker F, Khatamzas E, Rudawski IM, et al. Cytomegalovirus (CMV) phosphoprotein 65 makes a large contribution to shaping the T cell repertoire in CMV-exposed individuals. *J Infect Dis* 2002;185:1709-16.
3. Kern F, Faulhaber N, Frommel C, Khatamzas E, Prosch S, Schonemann C, et al. Analysis of CD8 T cell reactivity to cytomegalovirus using protein-spanning pools of overlapping pentadecapeptides. *Eur J Immunol* 2000;30:1676-82.
4. Bitmansour AD, Waldrop SL, Pitcher CJ, Khatamzas E, Kern F, Maino

- VC, et al. Clonotypic structure of the human CD4⁺ memory T-cell response to cytomegalovirus. *J Immunol* 2001;167:1151-63.
5. Fujimura T, Yamanashi R, Masuzawa M, Fujita Y, Katsuoka K, Nishiyama S, et al. Conversion of CD4⁺ T cell profile from T(H2)-dominant type to T(H1)-dominant type after varicella-zoster virus infection in atopic dermatitis. *J Allergy Clin Immunol* 1997;100:274-82.
 6. Roback JD, Hillyer CD, Drew WL, Laycock ME, Luka J, Mocarski ES, et al. Multicenter evaluation of PCR methods for detecting CMV DNA in blood donors. *Transfusion* 2001;41:1249-57.
 7. Khan N, Shariff N, Cobbold M, Bruton R, Ainsworth JA, Sinclair AJ, et al. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. *J Immunol* 2002;169:1984-92.
 8. Illi S, von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001;322:376-7.
 9. Laske N, Volk HD, Liebenthal C, Grüber C, Lau S, Sommerfeld C, et al. Infantile natural immunization to herpes group viruses is unrelated to the development of asthma and atopic phenotypes in childhood. *J Allergy Clin Immunol* 2002;110:811-3.

doi:10.1067/mai.2003.1548

Efficacy of montelukast, in combination with loratadine, in the treatment of delayed pressure urticaria

To the Editor:

Delayed pressure urticaria (DPU) is a type of physical urticaria characterized by the gradual onset of wheals or edema in areas where pressure has been applied to the skin. Usually, antihistamines alone are insufficient to control DPU; accordingly, corticosteroids might be beneficial to all patients with this disease, though it is associated with undesirable side effects. Montelukast is a cysteinyl leukotriene receptor (CysLT1) antagonist. Cysteinyl leukotrienes—LTC₄, LTD₄, and LTE₄—are strong smooth muscle constrictors and contribute to the onset of inflammation by causing plasma extravasation and eosinophil recruitment.¹ CysLT1 antagonists, given concomitantly with H₁-receptor antagonists, have been shown to be effective in the treatment of chronic asthma and chronic urticaria.^{2,3} The objective of our study was to evaluate the efficacy of the combination of montelukast and loratadine as therapeutic agents for DPU.

Twenty subjects (5 male and 15 female) affected by DPU were recruited. They ranged in age from 18 to 68 years (mean age, 45.5 ± 15.17 years). One month before treatment, patients were asked to discontinue systemic corticosteroids. The study was approved by the Ethical Committee, and each subject signed an informed consent form. Exclusion criteria were as follows: inability to understand the nature and scope of the study and/or evidence of an uncooperative attitude; cardiac, renal, hepatic, or rapidly progressing fatal disease; a history of alcohol consumption or hypersensitivity to montelukast or loratadine; pregnancy; pregnancy potential; current lactation.

After a careful history was taken and an accurate physical examination was performed, a challenge test was used to confirm the diagnosis of DPU. For the diagnosis of DPU, a 7-kg weight was suspended from the patient's shoulder for 15 minutes and readings of the test area were done at 0.5, 3, 6, 8, and 24 hours. Results were graded on a scale of 0 to 3, as follows: 0, severe response; 1, mod-

erate response; 2, mild response; 3, no response.⁴ The study was conducted in a randomized, double-blind fashion; results were evaluated on an intention-to-treat basis.

After diagnosis, each patient was randomly assigned to receive either loratadine 10 mg (n = 10) or loratadine 10 mg plus montelukast 10 mg (n = 10) for 15 days. At randomization, the baseline characteristics of the 2 groups were matched. Pressure-testing was repeated at baseline and after treatment and evaluations were made at each visit by the same investigator for each patient. Blood tests were performed (total blood cell count and blood chemistry [aspartate transaminase, total bilirubin, creatinine and urea]) at baseline and at the end of the study. Statistical analysis was performed with the Fisher exact test for 2 × 2 contingency tables (0-2, positive response; 3, no response). A probability of *P* < .05 was taken as statistically significant.

All 20 patients completed the study. Twelve patients (60%) had concurrent chronic idiopathic urticaria and/or dermatographism.

In this study, after challenge testing had been carried out at the end of the treatment, montelukast + loratadine was significantly more effective (*P* < .05) than loratadine alone. In fact, rechallenge testing revealed a total suppression of response after 3 to 6 hours in 8 patients (80%) from the montelukast + loratadine group and after 6 hours in 2 patients (20%) from the loratadine group. In contrast, 1 patient in the loratadine group and 1 patient in the montelukast + loratadine group had a moderate response, whereas 7 patients in the loratadine group and 1 patient in the montelukast + loratadine group had a mild response or no response. Blood chemistry tests conducted at the end of treatment revealed no changes in any of the studied patients, and no serious adverse effects were observed by the investigator or reported by the patient, confirming the tolerability of montelukast and loratadine. Somnolence was reported only in 2 patients receiving montelukast + loratadine.

The pathogenesis of DPU remains unclear. Characterized by a late-phase-type reaction occurring several hours after the application of weight, DPU is a disorder in which lymphocytes, eosinophils, mast cells, and mediators play an important role. Other authors have hypothesized that leukotrienes (particularly LTB₄) might play a role in such reactions.⁵ Increased levels of LTE₄ were observed in patients with cold urticaria and atopic dermatitis,^{6,7} and injection of the CysLTs into human skin produced a wheal and flare reaction.⁸

The anti-inflammatory potential of anti-leukotrienes in asthma has been confirmed in a number of studies.^{2,9} In vitro, it has been demonstrated that loratadine induces a reduction in LTB₄ levels¹⁰; in vivo, loratadine has been shown to inhibit histamine release and to decrease eosinophils counts in blood and sputum.¹¹ CysLT1 antagonists, such as montelukast 10 mg, have been shown to be effective with or without antihistamines in patients with chronic urticaria.^{3,12} The effect of leukotriene antagonists in the control of chronic urticaria has not been extensively studied.