Tumor necrosis factor (TNF)-α is an integral part of the immune response to infection. A number of polymorphisms in or near the TNF-α gene are associated with variability in TNF-α production. The three polymorphisms with the best documented in vivo and in vitro effect on TNF-α production are the TNF-α-238, TNF-α-308, and lymphotoxin (LT)-α + 250 loci. At each locus, a G to A transition is associated with greater TNF-α production. We hypothesized that the A alleles of these three polymorphisms would be associated with greater mortality and a greater risk of septic shock in patients with community-acquired pneumonia (CAP).

A prospective cohort study of 272 patients with CAP was performed with genotype determined by polymerase chain reaction amplification and restriction enzyme digestion. Among these, 24 patients (8.8%) died and 28 patients (10.3%) had septic shock. Genotype distribution was as follows: TNF-α-238 AA, 0 (0%); GA, 27 (10.3%); GG, 244 (89.7%); TNF-α-308 AA, 19 (7.0%); GA, 64 (23.5%); GG, 189 (69.5%); LT-α + 250 AA, 82 (30.1%); GA, 133 (48.9%); and GG, 57 (21.0%). Linkage disequilibrium existed between the LT-α + 250 A allele and the TNF-α-308 G allele (p < 0.001), and the LT-α + 250 A allele and the TNF-α-238 A allele (p = 0.01). Only the GA genotype at the TNF-α-238 locus was associated with increased mortality (25% vs 6.5%, p = 0.004; relative risk, 3.7; 95% confidence interval, 1.7 to 8.2). In a logistic regression model, TNF-α-238 GA genotype remained an independent risk factor for death (p = 0.02) with an age-adjusted odds ratio of 3.6 (range, 1.27 to 11.34). In contrast to mortality, a strong association between LT-α + 250 AA genotype and the risk of septic shock was found (relative risk, 2.5; 95% confidence interval, 1.3 to 1.8), with nonsignificant trends to greater septic shock with carriage of A alleles at TNF-α-238 and TNF-α-308. Analysis of TNF-α-308: LT-α + 250 haplotypes found the risk of septic shock to be associated with the G-A haplotype (p = 0.01), but not the A-A haplotype. Carriage of the TNF-α-308: LT-α + 250 G-G haplotype was protective from septic shock (p = 0.01).

In conclusion, the A allele at the TNF-α-238 locus is associated with a significantly greater risk of death in patients with CAP. AA genotype at the LT-α + 250 locus is associated with a greater risk of septic shock, but this locus is most likely to be a marker of another, causative polymorphism.

High and Low Inflammatory Response Phenotypes in 101 Normal Human Subjects*

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Abbreviations: IL = interleukin; VCAM = vascular cell adhesion molecule

Cytokine measurements in both BAL fluid and serum from patients with ARDS show a 10-fold to 100-fold difference. This variability likely explains why individual cytokines serve as poor prospective predictors of outcome in patients with ARDS and may partially explain why clinical trials of cytokine-directed inflammatory mediators largely have failed. Our goals were to determine the innate variability in the inflammatory response to a well-defined stimulus in healthy human whole blood and to identify patterns of cytokine production that correlate with high and low inflammatory response subsets of healthy subjects. An additional goal was to correlate these patterns with bioassays of net inflammation. Whole blood was obtained from healthy human volunteers (n = 101) and was stimulated with lipopolysaccharide (10 ng/mL) for 6 and 24 h at 37°C and 5% CO₂. Cytokine expression was measured via enzyme-linked immunosorbent assay. Endothelial cell vascular cell adhesion molecule (VCAM)-1 expression and neutrophil chemotaxis were used as different measures of net inflammatory activity. Assays were repeated serially in a subset of subjects and results were consistent and reproducible over four weeks. Tumor necrosis factor-α, interleukin (IL)-6, IL-1β, IL-8, IL-1ra, IL-10, and monocyte chemoattractant protein-1 production varied 5-fold to 10-fold. The net inflammatory activity in plasma varied 3-fold to 10-fold, and neither VCAM-1 induction nor polymorphonuclear leukocyte chemotactic activity correlated with any single cytokine response. High and low responders were identified as having cytokine responses 1 to 2 SDs from the mean. The data demon-

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Roles of Interleukin-13 and Interferon-γ in Lung Inflammation

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Abbreviations: IFN = interferon; IL = interleukin; Th = T helper

In human patients, asthma frequently is associated with mixed T helper (Th) cell responses. Th2 and Th1 cytokines (eg, interleukin [IL]-13 and interferon [IFN]-γ) can be detected in the lungs simultaneously. However, very little is known about critical mediators for the pathologic and physiologic changes that are induced by mixed T-cell responses in the lungs. Furthermore, IFN-γ has been reported to inhibit or promote Th2-induced lung injury.

To address this controversy, we examined an asthma model induced by mixed Th cell responses. IL-13 was a partial mediator of airway hyperreactivity and goblet cell hyperplasia, while effects on inflammation could not be detected. In contrast, IL-13 was critical for airway physiologic changes and inflammation in a Th2-induced asthma model that was examined in parallel, as has been reported before. In order to address whether the simultaneous presence of IL-13 and IFN-γ altered the responses in the lungs of the mice that had mixed T-cell responses to the antigen, wild-type mice were given recombinant cytokines intranasally. IFN-γ inhibited IL-13-induced goblet cell hyperplasia as well as the accumulation of eosinophils and neutrophils in BAL fluid. At the same time, when compared to single cytokines IFN-γ and IL-13 potenti-ated peribronchial, perivascular, and alveolar inflammation, IL-6 levels, and the numbers of natural killer and antigen-presenting cells in the BAL fluid. Because of the dual effects of IL-13 and IFN-γ in the lungs, it is likely that additional inflammatory mediators are differentially regulated. To further address this important question, this highly standardized model will be analyzed by gene expression microarrays.

A Search for Linkage to Atopic Asthma in Candidate Regions in a Danish Population

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Allergic diseases affect approximately one fifth of the general population in the Western world, and their frequencies seem to be increasing. Asthma is one of the most common chronic diseases. Family and twin studies have shown that a genetic predisposition is a very substantial risk factor for developing asthma and other atopic diseases. The genes and mode of inheritance behind these complex diseases are still largely unknown. Numerous association studies have been performed trying to identify the genes that are involved in asthma and atopy, but there have been no clear-cut conclusions. Family-based linkage analysis enables the identification of disease genes without knowing the specific mechanisms behind the disease.

The purpose of this study was to identify the genes involved in asthma and other atopic diseases in a Danish population. Several clinical population samples are being used for this purpose. One hundred forty asthma sib-pair families that have been thoroughly investigated clinically are now being genotyped in candidate regions. The families are genotyped using 120 microsatellite markers spaced at approximate 3-cm distance in seven candidate regions. These candidate regions include regions on chromosomes 5q, 6p, and 12q and four regions not previously

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