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Conflict of interest statement
None declared.

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5 Ross JD, Maw R; Bacterial Special Interest Group, and the British Clinical Co-operative Group of the MSSVD. How is gonorrhoea diagnosed on the basis of a questionnaire and a clinical examination? Lancet 2002; 359: 1655–69.

Communicable Disease Surveillance Centre (K A Fenton MPH, E Rudd MSc, T Nicholas MSc) and Central Public Health Laboratory (P A Johnson PhD, D M Livermore PhD), Health Protection Agency, London, UK. Centre for Infectious Disease Epidemiology, Departments of Primary Care and Population Sciences and Department of Sexually Transmitted Diseases, Royal Free and University College London Medical School, London (K A Fenton); and Department of Infectious Diseases and Microbiology, Imperial College London, St Mary's Campus, London (C Ison PhD, I Martin PhD). Correspondence to: Dr Kevin A Fenton, HIV/STI Division, Health Protection Agency, Communicable Disease Surveillance Centre, 51 Colindale Avenue, London NW9 5EQ, UK (e-mail: kevin.fenton@hpa.org.uk).
Antigens

Skin prick test reactivity

weight/volume with 0·9% weight/volume sodium chloride), cod, soya bean, hazel nut, peanut, birch, mugwort, alder, six local grasses, cat, dog, and Dermatophagoides pteronyssimus allergen Der p I, (ALK, Abello, Denmark); and latex (Stallergens, Marseille, France) as previously described. The test was judged positive if a wheal of 3 mm or larger was seen in response to any of the allergens in the presence of an appropriate response to the positive control (10 g/L of histamine dihydrochloride; ALK) and no response to the negative control (allergen diluent; ALK). During the previous follow-up, we did double-blind placebo-controlled challenges to cow’s milk when symptoms, clinical signs, or skin-prick test responses had been suggestive of cow’s milk allergy. At 4 years, cow’s milk challenges were repeated if persistence of the cow’s milk allergy was suspected.

We assessed the concentration of exhaled nitric oxide as a marker of bronchial inflammation. The expired air with dead space was collected in bags by the tidal breathing method. The concentration of exhaled nitric oxide was assessed within 4 h after collection by a Sievers 280 chemiluminescence analyser (Sievers, Boulder, CO, USA). The mean concentration of exhaled nitric oxide in healthy age-matched children was 10·1 ppb (range 7·3-18·2) (Tuomas Jartti, personal communication). 80 of the 107 children participating at the 4-year follow-up cooperated in the collection. Since acute respiratory infectious diseases increase and inhaled corticosteroids reduce the composition of the bifidobacterium microbiota might be crucial for maturation of human immunity to a non-atopic state; infants later developing antigen-specific IgE antibodies have more clostridia and fewer bifidobacteria in their stools during the postnatal period than those who did not. Specific strains of the lactobacillus microbiota, including lactobacillus GG, generate anti-inflammatory interleukin 10 and transforming growth factor β, suggesting a mechanism by which the risk of atopic eczema could be decreased, since the concentration of antigen-specific IgE antibodies was not reduced, at least by age 4 years. In fact, the cytokine production pattern induced by intestinal microbiota might be strain-specific. The IgE-independent protective effect of bacterial components on atopic disease we identified might be mediated by activation of the innate immune system via evolutionarily highly conserved pattern-recognition receptors.

Thus, distinct causes and pathogenic mechanisms underlie the heterogeneous manifestations of allergy. The immunological effects of probiotic strains also vary, even within the same species. Since the probiotic approach was promising and safe, future studies should focus on detection of new potential successful probiotic strains to be applied in combinations when combating allergic diseases.

Contributors

We thank Eino Hietanen (deceased), Department of Clinical Physiology, Turku University Hospital, for supervision of eNO assays and Johanna Hirvitech-Koskelainen for participation in the follow-up of the participants. The study was supported by grants from the Academy of Finland and Turku University Hospital (EVO Fund). The funding sources had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

Conflict of interest

No conflict of interest was declared.

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**RESEARCH LETTERS**

Association between tuberculosis and a polymorphic NFKB binding site in the interferon γ gene

Manda Rossouw, Hendrik J Nel, Graham S Cooke, Paul D van Helden, Eileen G Hoel

Interferon γ is believed to be crucial for host defence against many infections. To test the hypothesis that a polymorphism in the gene for interferon γ (IFNG) is associated with susceptibility to tuberculosis, we did two independent investigations. In a case-control study of 313 tuberculosis cases, we noted a significant association between a polymorphism (+874A→T) in IFNG and tuberculosis in a South African population (p=0.0085). This finding was replicated in a family-based study, in which the transmission disequilibrium test was used in 131 families (p=0.0055). The transcription factor NFKB binds preferentially to the +874T allele, which is over-represented in controls. This preferential binding suggests that genetically determined variability in interferon γ expression might be important for the development of tuberculosis.

Lancet 2003; 361: 1871–72

Tuberculosis accounts for the largest number of deaths worldwide that are due to a soil infectious agent. Interferon γ is thought to be essential for the immune response to infection in man, but genetic variability in the interferon γ gene (IFNG) itself has not previously been associated with susceptibility to infectious disease in the general population. Indeed, the importance of the interferon γ response in tuberculosis is unclear because the pathogen seems to have evolved strategies to disrupt the activity of the interferon γ molecule.1

A microsatellite polymorphism in the first intron of IFNG has been associated with several autoimmune and chronic inflammatory conditions.2 One particular allele of this microsatellite (the 12 CA repeat) is associated with increased production of interferon γ in vitro, and with allograft fibrosis in recipients of lung transplant. This link suggests a functional role in vivo for either the microsatellite itself, or, more probably, a functional polymorphism in linkage disequilibrium with the 12 CA repeat. A single nucleotide polymorphism (+874A→T) is situated directly adjacent to the CA repeat region in the first intron of IFNG. There was an absolute correlation between the presence of the +874T allele and the 12 CA repeat allele. The +874A→T polymorphism lies within a binding site for the transcription factor NFKB and electrophoretic mobility shift assays showed specific binding of NFKB to the allelic sequence containing the +874T allele. Since this transcription factor induces interferon γ expression, the +874T and +874A alleles probably correlate with high and low interferon γ expression, respectively.

In two studies, we investigated the role of the +874T→A polymorphism in tuberculosis. In a case-control study, participants were unrelated, and from a community in which all members were of similar socioeconomic status, have the same ethnic background (South African coloured) and have a high annual incidence of tuberculosis.4 Informed consent was obtained from all participants, and the study was approved by the ethics committee of the Faculty of Health Sciences, Stellenbosch University (Tygerberg, South Africa). To look for the +874A→T polymorphism, we genotyped DNA samples from 313 patients and 235 controls older than 15 years with no history of tuberculosis. The affected group consisted of 241 patients in whom pulmonary tuberculosis was bacteriologically confirmed, and 72 patients with tuberculosis meningitis.

Allele frequencies were the same in both affected groups and they were therefore analysed as one group. Cases were screened for HIV-1 and excluded from analysis if positive. Women accounted for 60% of both the disease and control groups. The control group was in Hardy-Weinberg equilibrium, and the sample size provided at least 95% confidence and 80% power (Epi Info 2000 version 1.1) for an allele with an odds ratio of 1.65 or higher. Genotyping was done by PCR as previously described.5

Statistical analysis by the χ² and Fisher's exact tests showed a significant association between +874A→T and tuberculosis for both the genotype (p=0.017) and the allele frequencies (p=0.0095) (table 1). The +874T allele seemed to confer protection against the disease, since comparison of +874T carriers versus non-carriers shows a significant difference between the disease and control groups (table 1).

We designed a second, family-based study to test the findings of the first one and to control for any possible population stratification and other environmental factors. We did a transmission disequilibrium test (TDT) in 131 families in which one or more child was affected with tuberculosis, and had at least one parent. Families were from the same community as in the first investigation, with no duplication between the cases of tuberculosis analysed in both studies. Of 287 patients, 274 consented to HIV testing, of whom ten proved positive for HIV-1. Affected children were excluded if HIV-positive (2.4%). 65 families had both parents available, whereas 66 had only one parent. There were 44 families with more than one affected child. Paternity of all the siblings was checked with highly polymorphic microsatellite markers, and instances of non-paternity were excluded from analysis. Data for 612 individuals were analysed with TRANSMIT (version 2.5.2). This program

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>TB (n=313)</th>
<th>Control (n=235)</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>186 (59%)</td>
<td>111 (47%)</td>
<td>1.16 (0.86-1.56)</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>102 (33%)</td>
<td>98 (42%)</td>
<td>1.00 (0.70-1.41)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>25 (8%)</td>
<td>26 (11%)</td>
<td>0.95 (0.50-1.81)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>235</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Case-control study: association of +874A→T with tuberculosis