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patients from polymerized haemoglobin compared with that from red-cell haemoglobin in patients with sickle-cell anaemia. This difference could alleviate the deoxygenation of red-cell haemoglobin in the microcirculation. Sensitivity of sickle cells to deoxygenation suggests that patients with sickle-cell anaemia might benefit from haemoglobin solutions more than the modest increase in haemoglobin concentration would suggest. On the basis of the response in this patient, we propose that the potential role of haemoglobin solutions should be explored in the management of vaso-occlusive complications in patients with sickle-cell anaemia and alloimmunisation to red-cell antigens.

#### Contributors

All three authors collected and analysed data, and wrote and revised the manuscript.

#### Conflict of interest statement

None declared.

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Division of Haematology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY 10467, USA (J P Raff MD, C E Dobson RN, H M Tsai MD)

Correspondence to: Dr Han-Mou Tsai  
 (e-mail: htsai@montefiore.org)

## Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children

Roger P Lauener, Thomas Birchler, Jill Adamski, Charlotte Braun-Fahrlander, Albrecht Bufe, Udo Herz, Erika von Mutius, Dennis Nowak, Josef Riedler, Marco Waser, Felix H Sennhauser, and the ALEX study group

Children of farmers are at decreased risk of developing allergies. Results of epidemiological studies suggest increased exposure to microbial compounds might be responsible for this reduced risk. Alterations in adaptive immune response are thought to be the underlying mechanism. We measured expression of receptors for microbial compounds known to trigger the innate immune response. We showed that blood cells from farmers' children express significantly higher amounts of CD14 (0.96 vs 0.43,  $p=0.0013$ ), and Toll-like receptor 2 (0.11 vs 0.04,  $p<0.0001$ ) than those from non-farmers' children. We propose that the innate immune system responds to the microbial burden in the environment and modulates the development of allergic disease.

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Children who grow up on a farm, or who are in frequent contact with farm animals, are at reduced risk of developing allergic diseases.<sup>1</sup> The living environments of farming families contain higher concentrations of the bacterial cell-

wall component lipopolysaccharide (endotoxin) than those of non-farming families.<sup>1</sup> These findings lend support to the idea that the microbial burden is an important environmental factor conferring protection against the development of allergies.<sup>2</sup> Effects on the adaptive immune system, such as enhancement of T helper (Th) 1-like responses by microbial compounds, or modifications of Th2-like responses,<sup>3</sup> are believed to play a part in mediating such protection.

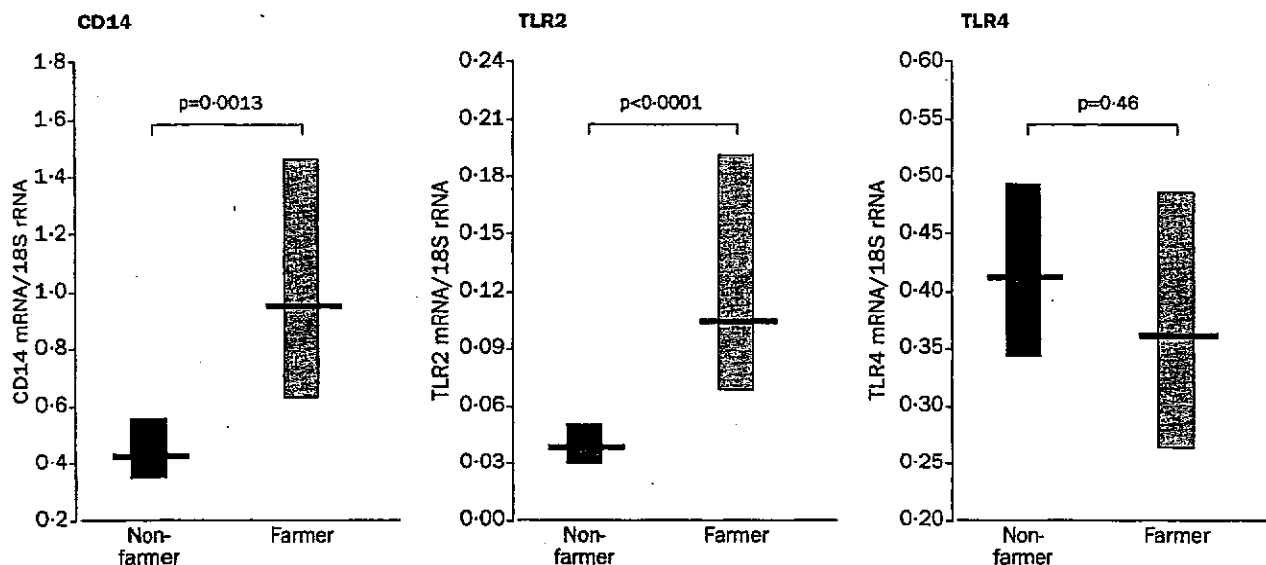
Innate immune responses are triggered through binding of lipopolysaccharide and other bacterial components to receptors such as CD14 and Toll-like receptors (TLRs). Human TLRs belong to a family of evolutionarily highly conserved proteins. Drosophila Toll protein was initially thought to play a part in embryonal pattern formation; later on, this protein was shown to mediate activation of the nuclear factor (NF)- $\kappa$ B homologue of Drosophila, and to initiate an innate immune response after exposure to microbes. Human TLRs, too, mediate cellular activation that involves translocation of NF- $\kappa$ B, and initiate an innate immune response when they recognise microbial compounds. TLR2 has an essential role in the response to compounds such as bacterial lipoprotein and leptospiral lipopolysaccharide; TLR4 mediates activation through lipopolysaccharide.<sup>4</sup>

In vitro, exposure of cells to lipopolysaccharide results in increased expression of CD14 and TLR2. We therefore measured the expression of CD14, TLR2, and TLR4 genes in blood samples from children exposed to high levels of microbial substances—ie, children of farmers—and in samples from children of non-farmers. In a previous cross-sectional survey we investigated the prevalence of allergic disease in farmers' and non-farmers' children in rural areas of Austria, Germany, and Switzerland.<sup>1</sup> The people in our study were the participants in the Swiss part of the cross-sectional survey from whom we had blood samples; this group consisted of 25 farmers' children and 71 controls. The local ethics committees approved the study. We compared our study sample to the source sample<sup>1</sup> for variables such as personal or familial history of allergy or asthma. The groups were mostly similar, but mean age was slightly higher in our sample (10.3 years) than in the source sample (9.6 years,  $p<0.0001$ ).

We assessed gene expression ex vivo with real-time quantitative PCR Taqman (Perkin Elmer Applied Biosystems, Rothkreut, Switzerland), without further stimulation of the samples in vitro. A description of the method and materials used is available upon request by e-mail from the corresponding author.

Expression of the CD14 and of the TLR2 gene normalised for the endogenous control (18s rRNA) was markedly higher in farmers' children than in non-farmers' children (figure, geometric mean CD14 mRNA/18S rRNA 0.96 vs 0.43, respectively,  $p=0.0013$ ; TLR2 mRNA/18S rRNA 0.11 vs 0.04,  $p<0.0001$ ). TLR4 expression was slightly reduced in farmers' children, but this difference was not significant (TLR4 mRNA/18S rRNA 0.37 vs 0.42,  $p=0.46$ ).

These differences in expression of CD14 and TLR2 in vivo parallel the effects of exposure of human blood cells to lipopolysaccharide in vitro, previously reported by other groups; the main effect of lipopolysaccharide in these investigations was also on expression of TLR2 rather than on the lipopolysaccharide-receptor TLR4.<sup>5</sup> The mechanisms underlying differential regulation of expression of TLRs have not been elucidated. We cannot draw any conclusions about the type of microbial compound eliciting receptor up-regulation, since components other than lipopolysaccharide—eg, bacterial lipoprotein—also alter the expression of TLRs.



#### Expression of the genes for CD14, TLR2, and TLR4 in children of farmers and in children of non-farmers

Data are amounts of mRNA specific for the target molecule (CD14, TLR2, or TLR4) normalised for amount of RNA of the endogenous control (18S rRNA). Geometric means and 95% CI are shown.

Exposure to microbes in the context of infections might have affected the expression of the molecules we assessed and thereby confounded our results. However, there were no signs of infection, such as fever, in any child at the time of blood sampling. Furthermore, leucocyte counts, which were done on all samples, were all within normal limits for age.

This study is a cross-sectional study assessing gene expression at the mRNA level. The next step would be follow-up studies to study receptor expression at the protein level and to assess expression of these molecules in individuals over time.

Our findings suggest that amplified gene expression of CD14 and TLR2 is related to increased environmental exposure to microbial compounds, and might therefore be used as a biological marker to indicate this type of exposure. Binding of microbial components to TLRs activates antigen-presenting cells, which could then either enhance a Th1-type immune response opposing the allergy-prone Th2-type immune response, or modify the Th2-type response.<sup>1</sup> We would expect such an instructive role of the innate immunity on the adaptive immune response to result in a reduced rate of sensitisation to specific allergens in farmers' children, which is the case.<sup>1</sup>

Additionally, the innate immune response may modulate the course of allergic disease through modification of the innate inflammatory response: immune mechanisms associated with lipopolysaccharide tolerance and not linked to allergen-specific sensitisation may become operative in an environment loaded with lipopolysaccharide and other microbial products. Such mechanisms might contribute to the protective effect against clinically manifest asthma conferred by the farming environment, which has proved independent of allergic sensitisation.<sup>1</sup> Irrespective of the nature of the subsequent immune reactions, the first step in a cascade of events could be critical.

Our findings raise the question whether the strong protective effect of being raised in a farming environment, especially early in life, is mediated by the innate immune system. Interactions between genes and environment are likely to happen at the level of the immune system, and may have a great effect on the risk of development of childhood asthma and allergies.

#### Contributors

R P Lauener initiated and designed the study, analysed results, and wrote the paper. T Birchler and J Adamski did PCR analyses and took part in analyses of results. A Bufer and U Herz participated in analyses of results and manuscript writing. M Waser participated in study design and analyses of results, and did field work. C Braun-Fahrlander, E von Mutius, D Nowak, J Riedler, and F H Sennhauser designed the ALEX study, did analyses of results, and wrote the paper.

#### Conflict of interest statement

None declared.

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Zurich University Children's Hospital, CH-8032 Zürich, Switzerland (R P Lauener MD, T Birchler MSc, J Adamski MSc, F H Sennhauser MD), Institute of Social and Preventive Medicine, University of Basel, Switzerland (C Braun-Fahrlander MD, M Waser MSc), Experimental Pneumology, Ruhr University, Bochum, Germany (A Bufer MD), Hospital of the Philipps University, Marburg, Germany (U Herz MD), Dr von Hauner Children's Hospital, University of Munich, Germany (E von Mutius MD), Institute of Occupational and Environmental Medicine, University of Munich, Germany (D Nowak MD), Paediatric Pulmonology and Allergology, Children's Hospital Salzburg, Austria (J Riedler MD)

Correspondence to: Dr Roger Lauener  
(e-mail: rlauener@kispi.unizh.ch)