A MARKED SHIFT IN INNATE AND ADAPTIVE IMMUNE RESPONSE IN CHINESE IMMIGRANTS LIVING IN A WESTERN ENVIRONMENT

To the editor:

We have previously reported that in comparison to their counterparts that have recently arrived from China, Chinese immigrants living in Australia for several years displayed attenuated innate cytokine responses following triggering of most TLRs, except for TLR-4. These immigrants also had overall increased methylation and decreased gene expression, which are consistent with recent findings suggesting that allergy-contributing factors such as microbial exposure, diet and environmental agents mediate their influence through alterations of the epigenetic landscape. Delayed maturation of the immune system with decreased production of IFN-γ at birth (Th1) and a prolonged postnatal Th2 deviation is associated with the development of allergy in childhood. The impetus for normal postnatal maturation of immune function is exposure to environmental microbial signals, and it is possible that differences in immune competence between recently arrived versus long-term Australian resident Chinese populations may reflect variations between the diversity and intensity of baseline microbial exposures in the respective environments.

To investigate these issues further, we extended our comparative studies on these populations to IgG-associated responsiveness to specific microbial pathogens that have been implicated in the pathogenesis of atopic asthma, and also to the correlation between their IgG production and their respective innate cytokine responses.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13531

This article is protected by copyright. All rights reserved.
Sera and whole blood was collected from 22 newly-arrived (≤5 years in Australia) and 22 long-term (≥5 years in Australia) Chinese immigrants in Perth, Australia (see Table E1 and E1). Innate response was measured by culturing blood with six toll-like receptor ligands: [PAM3CSK4 (PAM), polyinosinic-polycytidylic acid (p: IC), lipopolysaccharide (LPS), resiquimod (R848), peptidoglycan (PGN), and muramyl dipeptide (MDP)] and measuring concentrations of 23 cytokines involved in innate immunity. IgG1 response to *Haemophilus influenzae* (P6), *Streptococcus pneumoniae* (PspC), Rhinovirus (RV) A, B and C was measured in sera for adaptive immune response (see online repository for detailed methods).

There were no significant differences in the species-specific titers for RV-C, RV-A and RV-B in long-term compared to newly-arrived Chinese immigrants (See Figure E1). The IgG1 binding to PspC was significantly lower in long-term than newly-arrived Chinese immigrants (p=0.019). P6 showed no significant different titers in long-term compared to newly-arrived Chinese immigrants (See Figure 1).

We measured levels of production of 23 cytokines in whole blood from the Chinese immigrants after stimulation with 6 individual TLR ligands (PAM, p: IC, LPS, R848, PGN, MDP), resulting in 138 cytokine measurements for each individual. We calculated the correlation between the IgG1 levels (to five microbial antigens: P6, PspC, RV-A, RV-B, RV-C) and the 138 cytokine measurements in newly-arrived and long-term Chinese immigrants separately. There were 690 correlation coefficients (rho) derived from each of these two groups. The Spearman’s rho was used to determine the strength of the relationship between innate and adaptive immune responses in newly-arrived and long-term Chinese immigrants, separately. We had a null hypothesis that there is no difference in the patterns of these correlations between newly-arrived and long-term Chinese immigrants and we tested the hypothesis using a paired sample T test. The difference in the 690 paired rho-coefficients between the newly-arrived and long-term groups was highly significant (p = 2.8e-21). 67% (462) of the 690 rho-coefficients were higher in newly-arrived than long-term immigrants. From the 690 rho-coefficient, there were 75 r-coefficients with p-value ≤0.05 either in newly-arrived or long-term immigrants. For these 75 rho-coefficient pairs, the difference between newly-arrived and long-term
was also highly significant \( (p = 1.1 \times 10^{-10}) \). 84\% (63) of the 75 rho-coefficients were higher in newly-arrived than long-term immigrants.

We conducted principal component analysis to extract the first PC for IgG1 levels to PspC, RV-A, RV-B and RV-C, representative of adaptive immune responses. The percentage of responders to P6 was only 27.3\% (n=6), and was therefore excluded from this analysis. This adaptive immune PC was correlated with the 23 cytokine PCs (representative of innate immune responses) for newly-arrived and long-term Chinese immigrants. The rho-coefficient values between the two groups was significant \( (p=0.002) \). Most (16/23) of the rho-coefficients were lower in long-term Chinese immigrants (See Figure 2). Newly-arrived immigrants had stronger positive correlations between innate and adaptive immune responses.

After living in a Western environment, Chinese immigrants have an increased risk of allergy and asthma\(^1,6\). Although the exact mechanism is unknown, we hypothesise that Chinese immigrants acculturating to the Western lifestyle have less exposure to immunostimulatory microbial signals, thereby disrupting key immunoregulatory mechanisms that mediate resistance to allergic diseases.

We have previously reported that innate immune function appeared to be more robust in newly-arrived immigrants, and wanes over time in the less stimulatory Western environment\(^1\). In the present study, we further investigated antibody responses to bacterial and viral antigens in this Chinese immigrant population. Consistent with our general hypothesis, newly-arrived immigrants had higher serum levels of IgG1 to *Streptococcus pneumoniae* (PspC) than long-term Chinese immigrants, indicating more intense pathogen exposure in the former. More interestingly, the correlation profiles between innate and adaptive immune markers differed between newly-arrived and long-term Chinese immigrants. In particular the overall intensity of innate cytokine response to TLR ligands and the correlations between the latter and adaptive (antibody) responses to pathogen-associated antigens were significantly stronger in newly-arrived Chinese immigrants, suggesting that the higher levels of microbial exposure in their previous environment had resulted in a tighter integration of adaptive and innate immune pathways relative to that seen in the long-term group.
Innate control of adaptive immunity is a well-established paradigm. The prominent difference in correlation between innate and adaptive immune markers in newly-arrived and long-term immigrants suggests a deviation in crosstalk between the innate and adaptive immune systems in different environments (Western vs Eastern). Newly-arrived Chinese immigrants had a stronger positive correlation between innate and adaptive responses. The stronger correlation of innate TLR cytokine production and adaptive immune responses in newly-arrived immigrants shown in our study may be indicative of the overall stronger relationship between innate and adaptive immune responses observed in newly-arrived Chinese immigrants as production of TLR pathway cytokines is known to induce T-cell responses and drive adaptive immune response. Our findings parallel those of McDade et al who demonstrate that less hygienic environments increase the diversity and frequency of microbial exposures which results in more frequent inflammation that play a central role in innate immune responses.

Our study on Chinese immigrants was able to construe the relationship of innate and adaptive responses in relation to the Western environment. Conducting the study in a paired-matched manner enabled controlling for several important confounders such as age and gender. Innate and adaptive immune responses were measured at the same time point and recruitment was conducted in the same season and at close time points for each pair. A potential limitation is the lack of data on immune responses in these immigrants prior to migration, however this is beyond the feasibility of the study. Another limitation is the small sample size. However, our study has evidently indicated the influence of the Western environment on immune adaptation in Chinese immigrants, which may be associated with the aetiology of allergy and asthma in developed countries. In conclusion, there is a marked shift in innate and adaptive immune responses as well as their correlation profiles in Chinese immigrants after living in a Western environment for several years, which may be related to the increased susceptibility to allergic disease observed in this population.
REFERENCES


Aarti Saiganesh, BSc¹,²
Belinda J. Hales, PhD³
Yuchun Li, PhD³
Pat G. Holt, PhD²
Peter N. Le Souëf, MD¹
Guicheng Zhang*, PhD¹,²,³,⁴,⁵,⁶

¹School of Paediatrics and Child Health, University of Western Australia, Perth, Australia
²Telethon Kids Institute, University of Western Australia, Perth, Australia
³Xinxiang Medical University, Henan, China
⁴School of Public Health, Curtin University, Perth, Australia
⁵Centre for Genetic Origins of Health and Disease, Faculty of Health Sciences, Curtin University, Faculty of Health and Medical Sciences, The University of Western Australia, and Royal Perth Hospital Medical Research Foundation
⁶Curtin Health Innovation Research Institute - Biosciences, Curtin University, Perth, Australia

*Corresponding Author: Guicheng Zhang brad.zhang@curtin.edu.au

ACKNOWLEDGEMENTS

The authors thank Lee Hazell for her expertise and technical assistance. We also thank Shu Chen and Emilija Filipovska-Naumovska for assistance with recruitment; and all the study participants. The study was funded by Telethon Perth Children’s Hospital Research Funds.

DECLARATION OF INTEREST

All authors declare no conflict of interest.
AUTHOR CONTRIBUTIONS

GZ, PNLS, BJH and AS designed the study. GZ, AS and YL recruited the subjects and samples. AS and BJH conducted the experiments. GZ, PGH and AS analysed the data. All authors discussed the results and edited the manuscript.