Medical research

Smoking's lasting effect on the immune system

Yang Luo & Simon Stent

It emerges from a study of human cells that smoking can influence certain immune responses to the same extent as can age or genetics. Smoking can alter the immune system in ways that persist long after quitting the habit. See p.827

When our bodies encounter pathogens such as bacteria and viruses, immune cells release molecules called cytokines to coordinate the body's defence mechanisms. These cytokines send signals to other immune cells to mount an appropriate response against the invading pathogens. The secretion of cytokines can vary among individuals and is influenced by both environmental and inherited factors. On page 827, Saint-André *et al.*[1] report their examination of data from the Milieu Intérieur project[2], a research initiative designed to study the variability in the immune system among 1,000 healthy individuals.

The authors systematically examined 136 variables that might contribute to differences in cytokine secretion. These factors related to socio-demographics, diet and lifestyle. The authors discovered that three factors in particular – smoking, a dormant (latent) infection by a type of virus called cytomegalovirus, and a measure of body weight called body mass index (BMI) – were the main contributors to variability in cytokine response, and had comparable effects to those of age, sex and genetics.

To measure the effect of immune responses quantitatively, the authors analysed the production of 13 disease-relevant cytokine proteins. These were assessed in blood samples tested *in vitro* by exposure to 12 different immune stimulations, such as proteins associated with microbial and viral infections (Fig. 1). These stimulations elicited reactions from both lines of immune defence – the faster, more general, innate defence, and the slower, more targeted and adaptive, memory-based defence – serving as indicators of the body's immune activities.

Among the environmental factors studied, the authors report that smoking-related variables showed the most statistically significant associations across immune stimulations. Smoking was found to exert a transient effect on immediate, non-specific, innate immune responses. Surprisingly, its enduring influence on specialized adaptive immune responses was found to persist well beyond smoking cessation.

To investigate how smoking leaves this lasting effect on the adaptive immune system, the authors specifically tested its link with epigenetic alterations – molecular changes that help our cells to 'remember' their specific roles and functions. The study reveals that the association between smoking and cytokines in the adaptive branch of the immune system is shaped by a specific epigenetic process called DNA methylation, which modifies DNA sequences in the nucleus. This process functions in a way that is similar to issuing cellular instructions, directing cells to either activate or deactivate the expression of particular genes. Smoking was shown to decrease the level of DNA methylation at specific sites that are related to the regulation of genes associated with signalling processes and metabolism in the body, causing altered levels of cytokines in response to immune challenges.

The authors did not identify any specific cellular mediators for the increased inflammatory response to stimulation of innate defences in smokers compared with non-smokers. Instead, the authors found a clear link between active smoking and an upregulation of the bacterially induced inflammatory cytokine CXCL5. This particular cytokine has a role in orchestrating the immune response and is associated with an increase in the level of the protein CEACAM6 in the bloodstream of active smokers. CEACAM6 is involved in inflammatory processes and immune regulation, and it has been proposed to represent a clinical biomarker of disease for multiple cancers[3]. The higher-than-usual level of CEACAM6 in smokers suggests a mechanistic involvement in the pro-inflammatory cascade, triggered by bacterial stimuli, that is associated with smoking.

Saint-André and colleagues' study not only provides a scientific basis for further promoting non-smoking and a healthy lifestyle, but also highlights two key aspects for future studies. First, it indicates a way to search for more-realistic disease-prevention measures, including the possibility of identifying new molecular signatures of interactions between environmental factors and diseases, such as those observed in smokers compared with non-smokers. Second, it emphasizes the dynamic and context-specific nature of gene and protein activities, underscoring the need

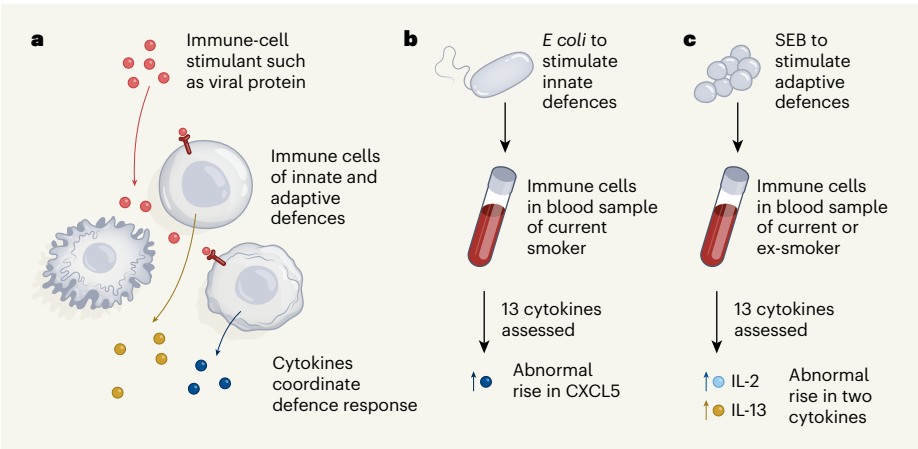

Figure 1 | Identifying factors that influence the production of cytokine molecules. a, When human immune cells encounter signs of problems they release various cytokines that orchestrate a defence response. Some immune cells function in the innate branch of the immune system, which provides a swift response to broad signs of infection. The other, adaptive, branch of the immune system enables highly specific targeting and generates a 'memory' of past infections. **b**, Saint-André *et al.*[1] examined cytokine production in response to immune-cell stimulants in blood samples from healthy people for whom 136 characteristics, such as smoking status, were known. The authors assessed whether any characteristics were associated with abnormalities in cytokine production. Current smokers had an impaired innate defence response to the bacterium *Escherichia coli* that was associated with a higher-than-normal level of the cytokine CXCL5. **c**, Current or ex-smokers had an abnormal adaptive response to a bacterial protein called *Staphylococcus aureus* enterotoxin B superantigen (SEB) that was associated with higher-than-normal levels of the cytokine proteins IL-2 and IL-13.

to understand disease-associated genes and proteins in their proper context. Although the authors' findings demonstrate the short-term and long-term effects of smoking on cytokine responses in healthy individuals, replication of the study in a clinical setting and with more genetically diverse populations would further aid understanding and modelling of these effects.

This work also highlights the importance of considering other environmental factors that can have both short-term and long-term effects on the immune system. Although some aspects of our immune responses are influenced by inherent factors that cannot be changed — such as age and genetics — other variables, such as smoking, BMI and viral infections, also have a key role in shaping human immune responses.

Taking a step back to consider the bigger picture, epidemiological studies have shown that environmental factors such as smoking and pollution are contributing to a global increase in the prevalence of cancer and cardiovascular and respiratory diseases[4]. However, there is still a lack of detailed understanding about the specific underlying cellular and molecular processes that are influenced by these environmental factors.

Saint-André and colleagues have shown that environmental exposures can affect immune responses associated with cancer through various mechanisms. These mechanisms include 'upstream' changes, such as DNA methylation, and 'downstream' effects on protein abundance. Epigenetic modifications and protein levels, such as those of CEACAM6, are therefore crucial for understanding how environmental exposures result in measurable immune responses. It will be essential to determine how environmental stressors affect epigenetic modifications, gene activity and protein function to better identify and mitigate the effects of environmental exposures on the immune system, and to understand the development of environmentally driven diseases.

Yang Luo is at the Kennedy Institute of Rheumatology, University of Oxford, Oxford OX3 7FY, UK. **Simon Stent** is a research scientist based in Oxford, UK.
e-mail: yang.luo@kennedy.ac.uk

1. Saint-André, V. *et al*. *Nature* **626**, 827–835 (2024).
2. Thomas, S. *et al*. *Clin. Immunol.* **157**, 277–293 (2015).
3. Burgos, M. *et al*. *Ther. Adv. Med. Oncol.* **14**, 17588359211072621 (2022).
4. Prüss-Ustün, A. *et al*. *BMJ* **364**, l265 (2019).

The authors declare no competing interests.
This article was published online on 14 February 2024.

Animal behaviour

How population size shapes fish evolution

Bernt-Erik Sæther

A long-term fish experiment reveals how a mechanism called density dependence, in which the population growth rate slows as the number of individuals rises, affects population dynamics on time scales relevant for ecology and evolution.

As populations grow, a decrease in their growth rate, occurring as a phenomenon referred to as density dependence, affects the dynamics of most species[1]. Writing in *The American Naturalist*, Travis *et al*.[2] provide evidence from guppy fish (*Poecilia reticulata*) on the island of Trinidad that sheds light on the wide-ranging consequences of this type of scenario. The authors demonstrate that variation in population fluctuations can lead to the evolution of large differences in life-history strategies in different populations affecting the pattern of survival of juveniles or adults, the timing of sexual maturity and the numbers of offspring produced.

This research delivers a key finding because most populations in the natural world are affected by this general feedback mechanism — the changes in population size from one point in time to the next depend on the number of individuals present in the population[3]. For nearly 100 years[4] it has been known from theoretical analyses that this type of internal feedback loop should have strong effects on the expected patterns of fluctuations in population size[5]. Density dependence is also known to result in natural selection of certain traits, (for example, the number of eggs produced per season by birds such as the great tit *Parus major*)[6], resulting in evolutionary consequences[7].

Yet, despite its general importance, experimental evidence from natural populations on how density dependence affects dynamic processes, on both ecological and evolutionary time scales, remains rare. Travis and colleagues' study of Trinidadian guppies fills a large gap in this lack of knowledge by experimentally demonstrating how patterns in the fluctuations in population size affect evolution through density-dependent selection, which affects variation in crucial characteristics of the life history of these fish.

The critical age-class[7] is a key concept in studies of evolution in density-regulated populations[8]. This a function that describes the age of individuals in a population at which the strongest regulation of population density occurs. A general prediction from theoretical analyses is that in density-regulated populations, evolution tends to maximize the expected value of the function that determines how the change in the number of individuals is affected by population size[7,8]. For example, the key variable affecting the density-dependent regulation of the size of a population might be either the total number or the total biomass of the individuals present[9].

Testing such effects of density dependence on life evolution in density-regulated populations requires that two conditions are fulfilled[8]. First, the stage of the life cycle that is most strongly affected by fluctuations in population size must be identified. Second, differences in 'fitness' of individuals in terms of the production of offspring (also described as recruits) by individuals must be closely associated with characteristics (phenotypes) that are present at this key stage of the life cycle. An exceptional feature of studying guppies is that they provide a unique opportunity to examine the validity of these key assumptions experimentally.

On Trinidad, guppies (Fig. 1) live in streams where they experience either high or low levels of predation from other species of fish. The composition of these predator communities was previously thought to be the primary selection pressure generating genetic differences underlying the life-history strategies of guppies, which relate to variation in the timing of sexual maturity corresponding to the age and size of the fish[10].

Nearly 15 years ago, the authors moved individual guppies from a high-predation location to generate four new experimental populations subject to two levels (high or low) of resource availability. Because the new populations were initially established using only a few individuals, monthly censuses provided precise estimates of the strength of density dependence. These included how the change in the number of individuals related to the population size; how fluctuations in