Credit: Maciej Frolow/Stone

AUTOIMMUNITY

Unravelling the complex genetic regulation of immune cells

Paula S. Ramos

Genetic variation contributes to immune cell function. An unprecedented analysis of genetic associations with immune cell traits provides insights into the complex regulation of immune cells, reveals variants that coincidently influence immune traits and autoimmune disease risk, and offers specific therapeutic targets for these diseases.

Refers to Orrù, V. et al. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. *Nat. Genet.* **52**, 1036–1045 (2020).

The diversity of the immune system is a result of both environmental and genetic variation. Although critical in host defense, this diversity also contributes to immune dysregulation such as that exhibited during autoimmune disease. Thus, to better understand immune system function and dysfunction, identifying the genetic and environmental factors that regulate variation of immune cell traits is important. In a large study published in *Nature Genetics*[1], a team led by Francesco Cucca identified multiple genetic associations with immune cell traits and coincident associations with autoimmune risk loci, thus linking immune trait variants to disease phenotypes.

Although the influence of age, sex, cytomegalovirus infection and smoking on immune repertoire variation is well documented[2], the contribution of genetic factors to this variation is only beginning to be elucidated. In this latest study, Orrù et al.[1] measured a total of 731 immunophenotypes in a family based cohort of 3,757 individuals from the founder population of Sardinia, including 539 immune traits profiled by flow cytometry (such as cell counts and median fluorescence intensities of cell surface antigens), and 192 relative counts. With these high-resolution immune data, the team estimated that the proportion of phenotypic variation of the immune traits due to additive genetic effects (that is, the traits' heritability) had a median value of 37.0%. They found higher heritability for lymphoid cells and those involved in adaptive immunity, especially naive cell subsets (up to 47.0% for naive T cells), than for myeloid cells and those involved in innate immunity, whereas the observed variation among mature and differentiated cells (for example, 29.3% for terminally differentiated $CD4^+$ T cells) seemed to be more strongly influenced by environmental exposures. These results confirm the contribution of genetic factors to variation in immune cell phenotypes.

> They found higher heritability for lymphoid cells and those involved in adaptive immunity

Five previous genome-wide association studies (GWAS) of immune cell traits were conducted to identify genetic variants associated with particular cellular immune phenotypes, resulting in the identification of close to 50 distinct loci associated with at least one immunological trait[3–7]. The study by Orrù et al.[1] greatly expanded on these findings by testing 22 million genetic variants for associations with 731 immune cell traits, unveiling 53 novel loci.

Given that the functional role of most genetic variants associated with immune-related diseases remains unknown, overlapping disease risk loci with immune cell trait loci might reveal 'coincident associations', thus suggesting potential causal relationships between a genetic variant, the involved immune cell subtypes and a disease. Of the 70 immune cell trait loci identified by Orrù et al.[1], 36 overlapped with reported GWAS disease risk loci. For example, an allele in the *SPATA48–IKZF1* region that was associated with decreased numbers of plasmacytoid

dendritic cells (pDCs) colocalized with an allele also associated with a decreased risk of systemic lupus erythematosus (SLE) and might thus have a role in the deregulation of pDCs in SLE. An investigation into the potential therapeutic utility of the findings suggested that downregulation of pDCs via inhibition of the pDC-specific receptor BDCA2 (also known as CLEC4C), whose expression is regulated by the DNA-binding protein Ikaros (encoded by *IKZF1*), is a promising therapeutic route for SLE. Indeed, as the authors indicate, an anti-BDCA2 monoclonal antibody that inhibits the production of type I interferon and other inflammatory mediators is currently in a phase II trial for SLE therapy[8].

> “multiple independent loci influenced the expression of a given surface marker in different cell subtypes”

In another example, an allele in *CD40* that is associated with increased expression of CD27 on memory B cell subsets overlaps with an allele associated with increased risk of various autoimmune diseases (such as SLE, multiple sclerosis and inflammatory bowel disease), as well as a decreased risk of rheumatoid arthritis and Kawasaki disease. This same allele was associated with decreased expression of CD40. Orrù et al.[1] found evidence implicating inhibition of CD27 on memory B cells as a therapeutic strategy in SLE, inflammatory bowel disease and multiple sclerosis. By comparison, current therapies for SLE and multiple sclerosis are based on broad depletion of B cells, rather than depletion of memory B cell subsets.

This study[1] clearly shows that the regulation of immune cell traits is complex. In some cases, multiple independent loci influenced the expression of a given surface marker in different cell subtypes with distinct effects on disease risk. For example, different independent variants at the *IL2RA* locus were associated with either higher or lower expression of CD25 in different cell subsets, and were associated with predisposition to or protection from different autoimmune diseases. Similarly, variation at the *CD28–CTLA4* locus was associated with reduced CD28 expression, especially in regulatory T cell subsets, whereas variants in *BACH2* were associated with increased CD28 expression in other T cell subsets. This intricate genetic regulation of immune cell levels and its consequences on immune-related diseases underscores the complexity of therapeutically targeting these diseases. Although most current biologic therapies for rheumatic diseases target a single protein, this study suggests that more efficacious and safer therapies ought to target multiple proteins to discriminate a particular cell subtype, or be based on targeted delivery of a drug to a specific cell type[1].

Despite the unprecedented number of immune cell phenotypes and genetic variants analysed, a limitation of this study[1] is the generalizability of the results to other groups and populations. The population of Sardinia is a founder population, which can help in identifying genetic variants that are rare or absent elsewhere but that occur at moderate frequencies in these populations. The discovery of new associations can elucidate causal mechanisms for immune phenotypes. However, it might be difficult to replicate such results in other populations because of the absence or rarity of the variant. In addition, nearly 80% of individuals in all reported GWAS are of European ancestry[9], which limits knowledge of genetic risk factors in ethnically diverse populations. This ‘information disparity’ affects the reliability of clinical genomic interpretation for under-represented populations[10] and can exacerbate health inequities[9]. The variation in prevalence of immune-related disorders along geographic gradients underscores the need to understand immune cell regulation in different populations and how the differences might affect the risk of disease.

Finally, this study[1] is a humble reminder that, despite extraordinary progress, much remains to be discovered about the genetic regulation of immune system variation. The effects of genetic and epigenetic variation, together with environmental exposures in individuals from different ancestries, must be elucidated for thorough understanding of the diversity of the immune system. The extensive data generated in this study[1] brings us closer to an improved understanding of the involvement of the immune system in human health and disease. This knowledge is expected to advance the field of medicine to use genomics in the transition to personalized medicine.

Paula S. Ramos

Departments of Medicine and Public Health Sciences, Medical University of South Carolina, Charleston, South Carolina, USA.

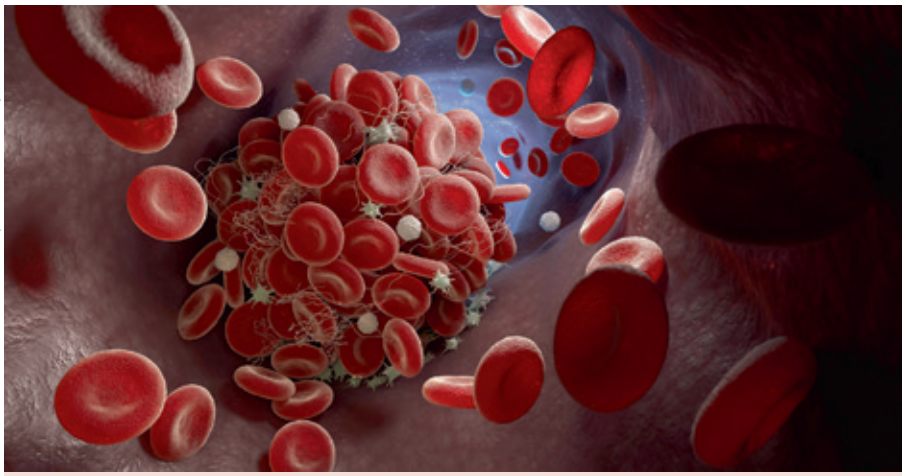
e-mail: ramosp@musc.edu

https://doi.org/10.1038/s41584-020-00563-1

1. Orrù, V. et al. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. *Nat. Genet.* **52**, 1036–1045 (2020).
2. Liston, A. & Goris, A. The origins of diversity in human immunity. *Nat. Immunol.* **19**, 209–210 (2018).
3. Aguirre-Gamboa, R. et al. Differential effects of environmental and genetic factors on T and B cell immune traits. *Cell Rep.* **17**, 2474–2487 (2016).
4. Orrù, V. et al. Genetic variants regulating immune cell levels in health and disease. *Cell* **155**, 242–256 (2013).
5. Patin, E. et al. Natural variation in the parameters of innate immune cells is preferentially driven by genetic factors. *Nat. Immunol.* **19**, 302–314 (2018).
6. Roederer, M. et al. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell* **161**, 387–403 (2015).
7. Lagou, V. et al. Genetic architecture of adaptive immune system identifies key immune regulators. *Cell Rep.* **25**, 798–810 (2018).
8. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02847598 (2020).
9. Sirugo, G., Williams, S. M. & Tishkoff, S. A. The missing diversity in human genetic studies. *Cell* **177**, 1080 (2019).
10. Popejoy, A. B. et al. The clinical imperative for inclusivity: race, ethnicity, and ancestry (REA) in genomics. *Hum. Mutat.* **39**, 1713–1720 (2018).

Acknowledgements
P.S.R. receives grant funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS; P30 AR072582) and the Scleroderma Foundation.

Competing interests
The author declares no competing interests.



Credit: Alexey Kotelnikov/Alamy Stock Photo

TARGETED THERAPIES

JAK inhibitors and VTE risk: how concerned should we be?

Stanley B. Cohen

Janus kinase (JAK) inhibitors have become standard treatment for patients with rheumatoid arthritis who do not respond well to other DMARDs. Concerns have been raised over an increased risk of venous thromboembolism with JAK inhibitors, tempering enthusiasm for their use in the clinic, but are these concerns justified?

Refers to Yates, M. et al. Venous thromboembolism risk with JAK inhibitors: a meta-analysis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41580 (2020).

Janus kinase (JAK) inhibitors have an efficacy similar to biologic DMARDs and are now widely used in the clinic to treat patients with rheumatoid arthritis (RA) with moderate-to-severe disease who have not responded well to conventional synthetic DMARDs. The adverse event profile of JAK inhibitors has been well delineated and, in general, is similar to that of biologic DMARDs except for an increased risk of herpes zoster[1]. However, concerns have been raised regarding JAK inhibitors and a potential increased risk of venous thromboembolism (VTE). The results of a new meta-analysis[2] of clinical trials of JAK inhibitors for immune-mediated inflammatory diseases is adding fuel to the debate about how concerned clinicians should be about the risk of VTE in patients taking JAK inhibitors.

In pooled analysis of the four phase III placebo-controlled trials of baricitinib in RA, an imbalance of the incidence of VTE was reported for the 4 mg daily dose (1.3 events per 100 patient-years) compared with the 2 mg daily dose or placebo (0 events per 100 patient-years)[3]. A VTE or pulmonary embolism signal was also noted in an FDA-mandated phase IV study evaluating patients with RA who had at least one cardiovascular risk factor, in which tofacitinib at 5 mg or 10 mg twice-daily doses were compared with standard doses of adalimumab or etanercept, with the primary end points of major adverse cardiovascular events or malignancy events[4]. In this study, a statistically significant increase in pulmonary embolism events occurred in those receiving 10 mg tofacitinib twice-daily and a numerical increase occurred in those receiving 5 mg tofacitinib twice-daily compared with patients treated with TNF inhibitors, and non-significant numerical increase in VTEs were reported for both doses of tofacitinib. By contrast, data from the tofacitinib RA clinical trial programme demonstrated no increased risk of VTE with incidence rates for 5 mg and 10 mg tofacitinib twice-daily of 0.29 and 0.28 events per 100 patient-years, respectively, similar to rates in published observational studies of patients with RA[4]. In the ulcerative colitis programme, the incidence rates for deep vein thrombosis and pulmonary embolism were 0.04 and 0.16 events per 100 patient-years, respectively, and all five events occurred in patients receiving the 10 mg tofacitinib twice-daily dose[5]. Data from clinical trials for upadacitinib and preliminary data from clinical trials for filgotinib have not demonstrated an increased risk of VTEs[6,7].

In their new meta-analysis, Yates et al. looked at phase II and phase III clinical trials of JAK inhibitors that have been approved for use in immune-mediated inflammatory diseases including RA, psoriatic arthritis, spondyloarthritis, psoriasis and inflammatory bowel disease[2]. Of the 42 studies evaluated, 29 were in patients with inflammatory arthritis. Yates at al. only evaluated the placebo-controlled randomized clinical trials for JAK inhibitors (tofacitinib, baricitinib, upadacitinib and filgotinib) and did not include the long-term extension protocols for these studies. For JAK inhibitors, 6,542 patient exposure years were evaluated compared with only 1,578 patient exposure years for placebo, which was as expected because the duration of placebo exposure in these trials was generally 8-12 weeks. Fifteen VTE events occurred in individuals who received JAK inhibitors and four in those who received placebo with incidence rates of 0.23 events per 100 patient-years (95% CI 0.12–0.38) for JAK inhibitors compared with 0.25 events per 100 patient-years (95% CI 0.07–0.73) for placebo[2]. On the basis of these results, the authors concluded that the pooled VTE risk for JAK inhibitors is unlikely to be increased compared with placebo and that the data do not support the current warnings around VTE risk for the typical trial participant who is offered a JAK inhibitor.

How does this meta-analysis inform the health-care provider? The top-line results are comforting, but the analysis is limited by the small number of events reported in the clinical trials and the limited overall exposure owing to analysis of only the placebo-controlled segment of these trials[2]. The majority of VTE events occur in the long-term extensions of the clinical trials[4], as would be expected with prolonged exposure to treatment. The inclusion of patients with psoriasis or spondyloarthritis, who tend to be younger than patients with RA and therefore might be at lower risk of VTE, could also affect the interpretation of the results. Patient level data were not available, and the effect of NSAID or glucocorticoid use and disease activity on VTE risk could not be assessed. In addition, patients with

> “mechanistic and observational data are still required to confirm or refute the role of JAK inhibitors in VTE risk”

substantial cardiovascular event or VTE risk factors, such as older individuals or those with a high BMI, are often excluded from such clinical trials. Therefore, the conclusion that VTE rates might not be increased for typical trial participants could be correct, but such individuals differ from the typical patient seen in the clinic, who often have multiple comorbidities and an increased risk of cardiovascular events or VTEs.

To better understand these conflicting data, we must acknowledge the fact that, in patients with immune-mediated inflammatory diseases including RA, the incidence of VTE is generally increased twofold compared with a matched control population[8]. Additionally, no mechanistic explanation currently exists as to how JAK inhibitors might increase VTE risk. In fact, by decreasing inflammation, one would think the risk should actually be decreased; for example, the selective JAK1 and JAK2 inhibitor ruxolitinib reportedly decreases risk of VTE in patients with polycythemia vera, who already have a high risk of thrombosis[9]. Patients with risk factors such as previous VTE, obesity, immobility or use of oestrogen replacement therapy have an increased risk of VTEs, and it is possible that JAK inhibitors increase the risk further in these patients. However, it is also possible that an increased number of VTE events could occur in patients treated with biologic or conventional synthetic DMARDs, although exposure to these therapeutics in the registration trials was too limited to address this question. Preliminary data also suggest that patients with RA who have high disease activity have an increased risk of VTE compared with those with disease in remission[10]. Patients treated with JAK inhibitors have generally been those with active disease that is refractory to other therapies, which could make the attribution of VTE risk to JAK inhibitors difficult.

Overall, the lack of risk noted in the meta-analysis by Yates et al.[2] is reassuring, but the question of JAK inhibitor safety in high-risk patients at the approved doses persists. Additional mechanistic and observational data are still required to confirm or refute the role of JAK inhibitors in VTE risk. At this point, and until additional data are available, we have a signal of concern about VTE risk but lack confirmation. As such, it seems appropriate to continue to follow regulatory recommendations to avoid JAK inhibitors in patients at increased risk of VTE if alternative therapies are an option. If alternatives are not available, a proper benefit-to-risk discussion with the patient is indicated.

Stanley B. Cohen[1,2]

[1]*Metroplex Clinical Research Center, Rheumatology Department, THR Presbyterian Hospital, Dallas, TX, USA.*

[2]*University of Texas Southwestern Medical School, Dallas, TX, USA.*

e-mail: arthdoc@aol.com

https://doi.org/10.1038/s41584-021-00575-5

1. Winthrop, K. L. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat. Rev. Rheumatol.* **13**, 234–243 (2017).
2. Yates, M. et al. Venous thromboembolism risk with JAK inhibitors: a meta-analysis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41580 (2020).
3. Genovese, M. et al. Safety profile of baricitinib for the treatment of rheumatoid arthritis over a median of 3 years of treatment: an updated integrated safety analysis. *Lancet Rheumatol.* **2**, E347–E357 (2020).
4. Mease, P. et al. Incidence of venous and arterial thromboembolic events reported in the tofacitinib rheumatoid arthritis, psoriasis and psoriatic arthritis development programmes and from real-world data. *Ann. Rheum. Dis.* **79**, 1400–1413 (2020).
5. Sandborn, W. J. et al. Venous thromboembolic events in the tofacitinib ulcerative colitis clinical development programme. *Aliment. Pharmacol. Ther.* **50**, 1068–1076 (2019).
6. Cohen, S. B. et al. Safety profile of upadacitinib in rheumatoid arthritis: integrated analysis from the SELECT phase III clinical programme. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218510 (2020).
7. Winthrop, K. et al. Integrated safety of filgotinib in patients with moderately or severely active rheumatoid arthritis receiving treatment for up to 5.5 years [abstract]. *Arthritis Rheumatol.* **72** (Suppl. 10), 229 (2020).
8. Choi, H. K. et al. The risk of pulmonary embolism and deep vein thrombosis in rheumatoid arthritis: a UK population-based outpatient cohort study. *Ann. Rheum. Dis.* **72**, 1182–1187 (2013).
9. Samuelson, B. T. et al. The impact of ruxolitinib on thrombosis in patients with polycythemia vera and myelofibrosis: a meta-analysis. *Blood Coagul. Fibrinolysis* **27**, 648–652 (2016).
10. Molander, V., Bower, H. & Askling, J. Does the risk of venous thromboembolism vary with disease activity in rheumatoid arthritis? [abstract]. *Ann. Rheum. Dis.* **79** (Suppl. 1), 23–24 (2020).

Competing interests

S.B.C. declares they have been a consultant and investigator for Abbvie, Eli Lilly, Gilead and Pfizer.