mechanisms underlie the beneficial effects exerted by graded sun exposure, which was used to treat chronic tuberculosis in sanatoriums at the beginning of the twentieth century. Interestingly, a systematic review of the period between 1971 and 2006 in 11 countries and regions from around the world confirmed a seasonal pattern of tuberculosis, with the most prominent peak during the winter and spring seasons (the time of the annual lull in vitamin D synthesis) in all of the countries studied.[7] Therefore, if infections represent one of the risk factors for developing early RA, the seasonality of the onset and severity of RA might be due to the seasonality of infections.

Secondly, when at normal serum concentrations, vitamin D downregulates T_H1-dependent immune responses, and its intake was found to be inversely associated with the risk of RA among patients.[6] Indeed, significantly lower ($P = 0.01$) serum levels of 1,25-dihydroxyvitamin D_3 were observed in patients with RA from the north versus the south of Europe.[8] A circannual rhythm of trough levels in winter and peaks in summer time showed, notably, significant negative correlation ($P < 0.0001$) with clinical RA status, as assessed using the 28-joint disease activity score.[8]

Recently, downregulation of Toll-like receptor 4-mediated production of IL-1β, IL-6, TNF, IFN-γ and IL-10 by peripheral blood mononuclear cells was observed in summer, compared with winter ($P < 0.05$), in healthy individuals.[9] Interestingly, after 3 months of high-dose oral therapy with the vitamin D_3 analog alfacalcidol, a positive effect on disease activity was observed in 89% of 19 patients with RA (45% achieved complete remission and 44%, 8 patients, experienced satisfactory improvement).[10]

In conclusion, the onset of arthritis symptoms during the winter or spring has been associated with greater radiographic evidence of disease progression at 6 and 12 months. That the seasonality of first symptoms might therefore act as an independent predictive factor of radiographic outcome and disease severity might be at least partially related to the seasonal lack of the immunosuppressive action exerted by vitamin D. If replicated in other early arthritis cohorts, the results of Mouterde *et al.*[1] might add further ways of assessing which patients should receive intensive early therapy for RA.

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The author declares no competing interests.

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SCREENING

CyTOF—the next generation of cell detection

Regina K. Cheung and Paul J. Utz

A new single cell detection technology allows simultaneous measurement of up to 100 surface markers and signaling proteins of immune cells. This method provides the opportunity to make great advances into the scientific understanding of rheumatic disease and the provision of individualized patient care.

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In the past decade tremendous progress has been made in understanding the roles of immune cell subsets in disease pathogenesis. This knowledge has led to the development of life changing new therapeutics, such as cytokine and co-stimulatory molecule antagonists, cell-depleting antibodies, and soon-to-be-approved small molecule kinase inhibitors. With these new treatment options come many unanswerable questions that rheumatologists have to face daily. Will this new drug work for rheumatoid arthritis? Will this patient be the one to develop an infection or a rare tumor? Will this drug also treat that patient's psoriasis, even though it is not approved for this indication? These questions highlight what is well known in the field: rheumatological diseases are highly heterogeneous within a 'disease', yet several diseases (for example, psoriasis, rheumatoid arthritis, and inflammatory bowel disease) seem to share genetic and pathogenic mechanistic underpinnings. A groundbreaking new technology, recently described by Bendall *et al.* in a paper published in *Science*,[1] has the potential to shed light on the mechanisms of rheumatic disease and, ultimately, help clinicians tailor treatment on an individual basis to provide the most effective care for each patient.

Fluorescence Activated Cell Sorting (FACS) is a tool commonly used in research laboratories to measure the abundance of immune cell types, and to quantitatively measure signaling activity in individual cells. Currently, the most common clinical users of FACS are hematologists and transplant physicians. Nevertheless, FACS also has rheumatological applications, such as diagnosis of the large granular lymphocyte (LGL) variant of Felty syndrome, and monitoring lymphocyte levels after rituximab treatment. To be analyzed by FACS, cells are stained with fluorescently labeled antibodies directed against cell surface or intracellular epitopes, which can include signaling markers, and passed in single file through an excitation laser. The laser excites the fluorophores, causing them to emit light at specific

wavelengths. The emitted light is measured, allowing identification of cell subsets, based on their expression of characteristic profiles of surface markers, and quantification of cell signaling using antibodies that recognize only the phosphorylated versions of the downstream substrates of kinases. However, FACS measurement is limited to around 10 markers because of spectral overlap between fluorescence emission profiles: emission of energy by each fluorophore occurs over a range of wavelengths and, therefore, one fluorophore may emit in the measurement range of another fluorophore, which would make it difficult and in some cases impossible to attribute the detected signal to a specific label.

The paper by Bendall *et al.*[1] describes single cell mass cytometry (CyTOF), an entirely new, more powerful technique that employs a unique method for detection of cellular epitope expression. CyTOF substitutes transition element isotopes for fluorophores as labels for antibodies, and—instead of the fluorophores being excited by laser light—the cells are vaporized and the isotopes are analyzed directly by a time-of-flight mass spectrometer. Using this technique, the authors demonstrate the ability to accurately measure as many as 34 markers simultaneously on every cell that is analyzed; up to 100 parameters will soon be possible, with no requirement for compensation of spectral overlap. To highlight the potential of this technique, Bendall and colleagues working in the lab of Garry Nolan performed immunophenotyping in the context of healthy human hematopoiesis using CyTOF, and identified differential signaling in distinct cell populations in response to various cytokines and kinase inhibitors.[1] As transition element isotope-tagged antibodies and CyTOF instruments become more widely available, this approach will be adapted to study essentially any disease, particularly rheumatic diseases in which circulating blood cells provide information on disease pathogenesis, and can be readily obtained and analyzed.

For instance, many cell types have been implicated in systemic lupus erythematosus (SLE) disease pathogenesis. B cells produce autoantibodies that can promote damage to organs such as skin and kidneys by activating complement and by binding to activating Fc receptors. Type I interferons, which are secreted at high levels primarily by plasmacytoid dendritic cells, have been implicated in SLE through the discovery of an "interferon gene signature" present in a large subset of adult and pediatric patients with SLE.[2,3] T cells are thought to provide help to B cells to induce full activation and isotype switching. Activated neutrophils may represent a source of autoantigens when they die or extrude 'neutrophil extracellular traps', or NETs.[4] CyTOF enables the study of all these cell types in mixed populations simultaneously. Until now, this has been difficult to achieve because of the limited number of cells available for study, and the limitations of FACS, particularly the small number of markers (~10) that could be studied in each experiment. Using CyTOF, it will be possible to broadly interrogate the quantity or physiology of each of these cell populations before, during and after a lupus flare, and to correlate these data with other multiplex assays, such as autoantibody profiles,[5,6] genetic polymorphisms,[7] as well as serum cytokine and chemokine levels.[8,9]

> "...isotope-tagged antibodies and CyTOF ... will be adapted to study essentially any disease..."

How the balance of cell subsets differs between groups of patients or within a single patient over time is important to understand; ultimately, the real prize is to understand how these subsets interact, and which are dysfunctional. CyTOF technology can be very powerful in this regard. Simultaneous measurement of distinguishing cell surface markers and phosphorylation of orchestrators of biochemical responses—such as signal tranducers and activators of transcription, phospholipase C-γ, nuclear factor κB and the mitogen activated protein kinases—allows for identification of cell subsets that might be signaling aberrantly. Analyses such as these will allow for observations of the off-target effects of current drugs, including steroids, TNF inhibitors or other biologic agents, and kinase inhibitors such as those being developed to inhibit the Jak family of kinases. Similar investigations using CyTOF might also enable identification of new pathways to target for drug development. For example, CyTOF might identify a new kinase pathway that is particularly dysregulated in a disease subset, or could show that an existing kinase inhibitor works best if a particular cell population is targeted.

CyTOF is unlikely to appear on the 'Boards' or to be ordered by practicing rheumatologists as a Clinical Laboratory Improvement Amendments (CLIA)-certified assay in the near future. However, CyTOF immediately provides the opportunity to perform vastly multiplexed single cell analysis that will aid identification of biomarkers, new pathways, and cell types that are dysregulated in rheumatic diseases. The CyTOF technique is particularly suited to defining subsets of patients with particular disease manifestations, and in predicting impending disease flares. The information gained from CyTOF investigations might help elucidate why drugs like rituximab and belimumab are efficacious in some but not all patients, potentially saving many millions of dollars by allowing current treatment regimens to be streamlined and refined. In time, CyTOF has the potential to bring systems biology to individualized medicine, thus helping clinicians understand when to use a TNF antagonist, a co-stimulatory blocker or a kinase inhibitor, not for groups of patients, but for each patient.

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The authors declare no competing interests.

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