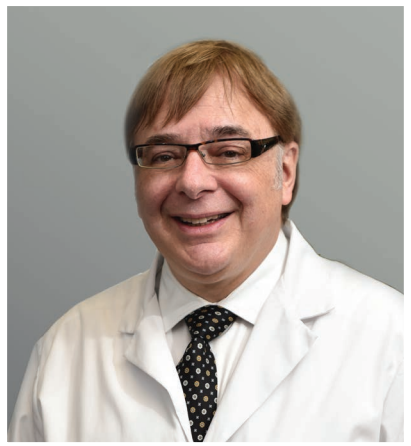
Establishing the Individual Immune Identity Card of Scleroderma Patients

An Essential First Step Toward Personalized Therapy



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At the request of several provincial scleroderma (Scl) associations, I am pleased to provide an overview of recent research carried out under the University of Montreal Scleroderma Research Chair.

For the past several years, an advanced program of translational research on Scl is in progress in Montreal at the Research Center of the Centre Hospitalier de l'Université de Montréal (CHUM). This program results from a close collaboration between the Laboratory for Immunoregulation, directed by Marika Sarfati, MD, PhD, an immunologist and basic scientist, and the Laboratory for Research on Autoimmunity, directed by the undersigned, a rheumatologist and clinician researcher specialized in the care of Scl patients.

THE RATIONALE FOR A PERSONALIZED APPROACH TO TREATMENT

These scientists are closely collaborating on an exciting and state-of-the-art novel Scl research project aimed at changing the often poorly efficacious "one size fits all" approach to therapy of Scl into ***personalized medicine***, i.e. the prediction of Scl progression in individual patients and predicting whether they will respond to drug treatment. The ultimate goal of personalized medicine is the use of therapies specifically developed and individualized to target the disease mechanisms specific to a given Scl patient. We and others believe that this approach is much more promising because it will be based on identification of the disease mechanisms that are present in individual Scl patients very early in Scl and that this will predict their disease course, thus allowing therapies to be tailored to the individual Scl patient.

Until recently, exploring these mechanisms was not possible other than on a small scale but exciting new (and costly) technologies are now available on a very large scale commensurate with the high cellular and molecular complexity of Scl.

THE NEED FOR EARLY THERAPIES IN SCL

But before explaining more in-depth our project and its potential high relevance to Scl patients, providing some essential background is needed. Scl is a potentially life-threatening and incurable disease. Although physicians do not like to admit it, ***most therapies in Scl (as in many other diseases) are given after the fact***, i.e. after a single or several organs are involved. For example, if moderate or severe lung fibrosis develops, attempts are made to treat with immunosuppressive and/or anti-fibrotic drugs, hoping at least to stabilize the patient's condition. Sometimes this is not possible and lung fibrosis progresses so much that lung transplantation is necessary, showing the limits of current medical therapies. There is a profound need to develop early and preventive therapies in Scl, i.e. treatments administered before the development of severe manifestations in the patients at-risk.

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THE CENTRAL ROLE OF THE IMMUNE SYSTEM

This lack of efficacy is not surprising in light of the extremely complex pathogenesis of Scl comprising four cardinal disordered mechanisms that ultimately lead to Scl symptoms through involvement of the skin and internal organs. Thus, ***autoimmunity*** (attack of the immune system against one's self leading for example to the production of autoantibodies in the blood that are highly specific for Scl) and ***inflammation*** combine with widespread ***involvement of small blood vessels*** (causing Raynaud's phenomenon, i.e. blanching of the digits on cold exposure) and ultimately ***fibrosis*** (excessive production of collagen leading to hardening of the skin and internal organs). Scientists have become aware that many cells (mononuclear phagocytes, fibroblasts, myofibroblasts, endothelial cells, vascular smooth muscle cells, to name but a few), hundreds of genes and hundreds of molecules (cytokines and many others) participate into Scl pathogenesis. ***Since inflammation precedes fibrosis, the immune system is at the core of the disease process and therefore its study is critical***. But how can such immune complexity be deciphered?

THE NEW CONCEPT OF PRESCLERODERMA

Two breakthrough discoveries by our research team started to answer this daunting question and provided major clues as to where to start. First, in 2008, we reported in the leading rheumatology journal worldwide that, in almost ***all patients with Scl, an immune signature of the disease (i.e. Scl-specific autoantibodies in the blood) is present many months and often years before manifestations typical of Scl are noted*** by the patient and her doctor (such as thickening of the skin or lung involvement) [(1)]. This discovery focused attention on a very important and previously neglected period in the Scl time course that is now universally designated as prescleroderma (preScl). Our discovery raised three key questions:

- 1° Since patients with preScl eventually develop full-blown Scl, what are the pathogenic disease mechanisms active in the preScl phase?
- 2° Can these mechanisms predict the future course of the disease and involvement of specific organs?
- 3° Can these mechanisms be targeted early by novel or existent therapies to change the future course of Scl?

However, at the time, advanced technology to explore in-depth the cellular and molecular mechanisms of early Scl was not yet available. Therefore we decided to tackle these questions by developing an experimental (animal) model of Scl for which Dr Sarfati is an expert. An animal model is useful because it allows analysis at a greater level of biological complexity, i.e. closer to humans, than is possible in the test tube. Also, it allows therapeutic manipulations that would be unethical in humans.

COPYING HUMAN SCLERODERMA INTO MICE

The successful development of this model was ***the second breakthroug***h. Said shortly, we succeeded into ***copying human Scl into mice***. Not only did our model replicate the four cardinal mechanisms of Scl, it also replicated the Scl phenotype, i.e. clinical manifestations such as skin thickening and lung involvement. Moreover, since we had previously identified preScl as a potential critical period for early therapeutic intervention, as seen above, we proceeded to therapeutic manipulations (antibiotic therapy to change the gut microbes) during the corresponding preScl period in the mouse model. The results were dramatic, indicating ***a profound effect on disease severity when therapeutic manipulation was applied specifically during preScl***. This discovery was published in 2017 in a top international dermatology journal with an accompanying editorial [(2)].

NATIONAL SUPPORT

I wish to emphasize that the development of the model, originally supported by Sclérodermie Québec, was also generously supported by donations from Maureen Sauvé, Scleroderma Canada, Scleroderma Society of Ontario and the Scleroderma Research Chair, which allowed us to generate strong preliminary results and to obtain a research grant from the Canadian Institutes of Health Research (CIHR). Thus, we used these private donations as leverage to obtain highly competitive public funding ($ 575,740, 2015-2019; success rate: 9%). Currently, we are supported by Sclérodermie Québec, the Research Chair and by donations from the Scleroderma Associations of British Columbia, Manitoba and Saskatchewan, emphasizing the national stature of our program.

PRESCLERODERMA: A NOVEL POTENTIAL THERAPEUTIC WINDOW

Taken altogether, these 2008 and 2017 discoveries, both in preScl patients and in the experimental model, ***identified preScl as a new and potentially very important therapeutic window*** for Scl patients. Therefore, it became critical to study in-depth at the cellular, molecular and gene levels patients with preScl in order to characterize early immune mechanisms, compare them with disease mechanisms active in full-blown Scl and identify potential novel therapeutic pathways and molecular targets. Remarkably, over the past few years, technological breakthroughs occurred that now permit analysis of disease mechanisms at an unequalled and extraordinary level of cellular, molecular and genetic complexity. >>

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THE FACSYMPHONY

Thus, in late 2018, the CHUM Research Center, with support from Sclérodermie Québec and the Canadian Foundation for Innovation, acquired a cutting-edge instrument from Becton-Dickinson (BD) called the ***FACSymphony, a multiparameter flow cytometer that allows complex molecular analyses at the single-cell level*** that were not possible previously. As many as 30 (and in the near future up to 50) molecular characteristics of a single Scl cell can now be identified using only limited amounts of blood and small skin biopsies (3 mm) from Scl patients, thus enabling to comprehensively study their immune profile in blood and at barrier tissues. Over the past several months, Dr Sarfati and Heena Mehta, PhD, Research Associate, have become experts at using the FACSymphony and its complex results consisting of big data that require artificial intelligence-based software and computational biology for interpretation. The power of this new technology is extraordinary and will lead to the identification of unsupected pathogenic cellular subpopulations and new disease mechanisms.

A GROUND-BREAKING RESEARCH PROTOCOL

Having mastered the FACSymphony, our expert team composed of Dr Sarfati, Dr Mehta, Sabrina Hoa, MD (a rheumatologist and clinician researcher who has completed a 3-yr Scl fellowship and a Masters in epidemiology at McGill University), Martial Koenig, MD (an internist specialized in Scl and the first author of the 2008 manuscript) and the undersigned undertook and are now completing, despite COVID19-related containment, ***a new research protocol with the aims of characterizing the overall immune landscape in Scl and preScl and establishing specifically "the immune molecular and cellular identity card" in individual Scl patients***.

The study design takes stock of our discoveries of preScl and our mastery of the unequalled analytical power of the FACSymphony. ***Results will provide important novel therapeutic targets, the inactivation of which may prevent Scl progression. We anticipate the potential discovery of a "protective" immune profile, that may suggest novel therapeutic targets and strategies to protect patients from progressing to full-blown Scl***.

THE RHAPSODY

However, a key instrument was missing to maximize the identification of novel potential therapeutic targets. ***Additional state-of-the-art technology was needed to provide transcriptomes (all the messenger RNA molecules) of the targets identified by flow cytometry. This equipment, also from BD, is the Rhapsody, a single-cell analysis system that provides the expression of hundreds of genes across tens of thousands of single cells in parallel***. Like the FACSymphony, with which it operates in tandem, the Rhapsody is expensive and requires access to highly valuable patient biological samples. Therefore we wished to optimize the number of analyses on the same sample as much as possible and this is what justified the rapid acquisition of the Rhapsody, as the same precious patient samples used in the FACSymphony can be reused in the Rhapsody. Moreover, we recently had the opportunity to test the potential of the Rhapsody through a loan of demo equipment by BD. ***Put simply, not having the Rhapsody would deprive our research project from its potential therapeutic applications***.

A SUCCESSFUL NATIONAL EFFORT

Given that the CHUM Research Center did not own a Rhapsody and that our team did not have the financial resources for its purchase, Sclérodermie Québec launched a national fundraising appeal. We are extremely pleased that $88,000 were raised in March and April 2021, that will allow the purchase of the Rhapsody. We thank for their generosity Scleroderma Canada, the Scleroderma Association of B.C., the Scleroderma Association of Saskatchewan, Scleroderma Manitoba, the Scleroderma Society of Ontario, Sclérodermie Québec and Scleroderma Atlantic.

CONCLUSION

Technological advances are now providing extraordinarily powerful tools to establish the individual immune identity card of Scl patients. As we are taking our first step toward personalized therapy, these tools raise hope for all Scl patients. Partnerships between patients, basic science researchers and clinician researchers, and generous support from provincial and national organizations are critical to improve the life expectancy of Scl patients, and ultimately find a cure for Scl. Our team is proud of the national support for its projects. We are accountable and we deliver. <<

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1. Koenig et al. Arthritis Rheum (2008) 58: 3902-3912. doi: 10.1002/art.24038.
2. Mehta et al. J Invest Derm (2017) 137:2316-2325. doi: 10.1016/j.jid.2017.06.019.