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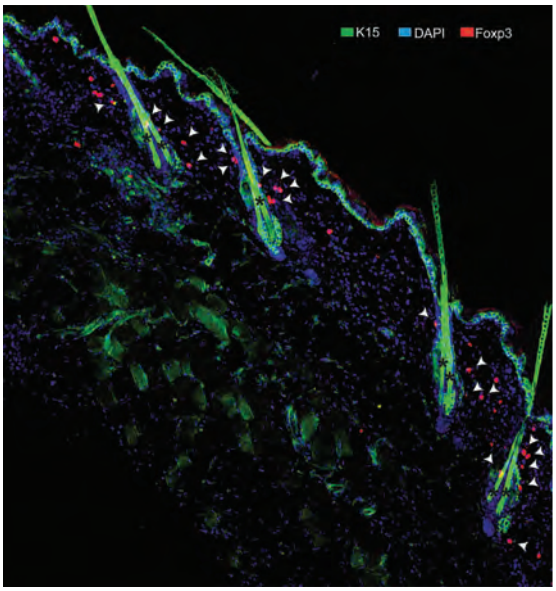
DERMATOLOGY FOCUS™

Skin-Resident Tregs:

A Powerful Multi-tasking Force for Immune Balance and Tissue Renewals

Michael D. Rosenblum, MD, PhD, associate professor in the department of dermatology at the University of California, San Francisco, is both a practicing dermatologist and an immunologist. He has been making startling discoveries about regulatory T cells—*Tregs*—in the skin that initially took him by complete surprise. He had realized that new information would emerge simply because his areas of interest—immune regulation rather than activation, and in the affected tissue (skin, in this case) rather than in lymphoid structures—had not yet received serious research attention. But Rosenblum was not prepared for results that upended conventional concepts of T-cell immunology.



Tregs and hair follicle stem cells. Immunofluorescent close-up of Foxp3+ Tregs *(red stain, with white arrows)* shows subpopulation of bulge-associated Tregs closely aligned with HFSCs in mouse telogen skin. (Green-stained hair keratin K-15 is expressed in the hair follicle above the bulb; DAPI is a fluorescent blue stain.) (Reprinted with permission from *Cell*. See *Suggested Readings* for citation.)

Choosing His Focus

Rosenblum's passion for immunology had been sparked during college in the late 1990s, and crystallized when he came across *The Transformed Cell: Unlocking the Mysteries of Cancer* by Dr. Steven A. Rosenberg, the innovative cancer immunologist, surgeon, and melanoma specialist at the NCI who has developed several personalized anti-cancer immunotherapies. Rosenblum modified his direction from scientist to physician-scientist, ultimately choosing dermatology. The skin is prone to a number of inflammatory and autoimmune diseases. And as our major barrier to the outside world, all of the skin's protective mechanisms clearly interface with the immune system.

When Rosenblum began his post-doctoral research (working with world-renowned UCSF immunologist Abul K. Abbas, MD) that launched his groundbreaking series of discoveries, he took a less well-traveled road and earmarked Tregs within the skin for his primary study. Tregs express a protein called Foxp3, which is unique to these cells and critical in regulating inflammation. When Foxp3 had been discovered in 2001—also called *scurfin* then—it became apparent that it binds the DNA of inflammatory cytokines and represses their transcription. And loss of Foxp3's function due to mutations was associated with significant autoimmune disease in mice and humans.

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Focus on Research

Adipose Stem Cells—Understanding Them and Their Potential Value for Aesthetic and Regenerative Medicine

Ivona Percec, MD, PhD

Assistant Professor of Surgery, Department of Surgery, Division of Plastic Surgery; Director, Basic Science Research, Plastic Surgery; Perelman School of Medicine, University of Pennsylvania

Dr. Percec always knew she would be a clinician and a scientist. She chose plastic surgery as her clinical specialty because she loves the combination of working with her hands, repairing aesthetic and relevant functional deficits, and the challenging variety of problems and body areas that she is able to address. And when she joined the faculty at the University of Pennsylvania, she established her lab and dedicated it to exploring human autologous fat grafts—her favorite multi-tasking tool in plastic surgery—at the molecular level.

She is fascinated with autologous subcutaneous fat, which entered the surgical toolbox as a corrective filler in 1893 (see box on page 14). "It has so many advantages, so many clinical applications and beneficial effects, that in the operating room we call it *liquid gold*," she says. Access is easy. Supply is abundant. Adipose stem cells (ASCs) are high in number without requiring special

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2017: AC *Sustaining* Generosity Continues to Grow

AC *Sustaining* members have each made the significant decision to contribute $5,000 annually after completing their $25,000 AC commitment. The Dermatology Foundation offers a special *thank-you* to those who chose to become a *Sustaining* member in 2017, and to *Sustaining* members who chose to extend their *Sustaining* commitment. This exceptional generosity strengthens their support of scientific and clinical progress well into the future. *Sustaining* members are recognized acccording to their cumulative AC giving level.

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"Most of the seminal work in immunology, though, focused on activating an immune response, on how to create an immune response against something like cancers and infections," Rosenblum explains. "Since activation occurs in lymphoid structures, a lot was learned about the activation dynamics there. But I had appreciated very early on," he continues, "that many times when we want the immune system *not* to respond—it often not only responds anyway, it responds too vigorously. Autoimmune diseases are a prime example. Yet we know very little about how immune responses are controlled in the tissues where they actually occur," Rosenblum adds. Few had studied how immune responses are controlled in peripheral tissues such as skin—until Rosenblum made this his goal.

He turned his attention to Tregs in the skin—both human and mouse. And he wanted to find out what they do there, how they do it, what can go wrong—and how they get there in the first place. "We've been finding that these cells do a number of things to regulate the immune system and inflammation in the skin," Rosenblum says. "But some of the most high impact work we have been able to do over the last several years is to show that these cells actually have other functions—functions outside of their ability to suppress immune responses. And this was previously either not known, or not very well appreciated. These cells are multi-taskers," Rosenblum states. Ultimately this should lead to knowledge that can be exploited for therapeutic benefit.

Mouse vs Human

Overall, the human relevance of data gathered from studying mice can be as low as 8% of observations. Against this general caution, it is clear that human skin and mouse skin are profoundly different, and most dramatically when it comes to the hair cycle. It is brief, rapid, and synchronous for mice, and very much the opposite for humans.

Keenly aware of this reality, Rosenblum views his mouse data as provisional until he has either validated it in human tissue or determined that humans are different. He tries to keep his research moving in the right direction by "going back and forth between mouse and man." They usually begin with human tissue samples, and once they have discovered pathways that they think are important in human disease, they do the functional biology in mice as it is impossible to do this in humans outside of a clinical trial.

A major focus in Rosenblum's laboratory now is the development and use of humanized mouse models. They are genetically modified to be selectively immunodeficient so that they will accept human tissue and a human immune system. This enables the study of human immune responses using mice, and Rosenblum says that "they are proving to be a valuable tool for modeling human autoimmunity and chronic inflammation. The basic immunobiology of human tissue is readily studied in these models."

The lab has evolved to the point where their study of the regulatory immune system has achieved a rough balance, with studies using human and mouse tissue split roughly 40% vs 60%. Although most of Rosenblum's publications until now are based on observations in mice, he has "a flurry of publications scheduled to appear that contain a substantial amount of human data—the major pathways that we discovered in human skin and then have been modeling in mice."

Regulatory Memory Cells Are Permanent Residents in the Skin

Immune homeostasis in tissues is achieved through a delicate balance between pathogenic T-cell responses directed at tissue-specific antigens and the ability of that tissue to inhibit these responses. But the mechanisms by which tissues and the immune system communicate to establish and maintain immune homeostasis had been a mystery. That something is going on in the tissue to

diminish pathological inflammatory responses is suggested by the attenuation of symptoms in repeated flares of autoimmune diseases and by the desensitization process when specific allergens or self-antigens are repeatedly injected in the skin.

To study how immune responses are regulated in skin, Rosenblum needed a mouse model that allowed him to set the stage, then watch to see what would happen. He needed the ability to introduce an immunostimulatory self-antigen only in the skin, and then be able to turn its presence on and off at will. He wanted to bring this new self-antigen silently into the skin, turn it on to see how the initial immune response to it is generated, then turn it off to see if the meter resets to zero or not, turn it on again to see if it's simply a repeat of the initial response or something different, and repeat this to see how the immune response is regulated over time.

Michael D. Rosenblum, MD

Rosenblum engineered a mouse using ovalbumin (Ova)—the major protein constituent of chicken egg whites and a standard antigen for immunization research—as the self-antigen, as this glycoprotein is sufficiently large and complex to be mildly immunogenic. He was able to mimic the pattern of tissue-specific self-antigen expression in mice and humans, maintaining the continuous presence of Ova in the thymus while turning it on and off in the skin. Ova delivered to the skin carried a tetracycline-activated receptor, so the antigen appeared when tetracycline was added to the mouse food and disappeared when the tetracycline was stopped.

The initial presence of Ova induced a pronounced inflammatory dermatitis despite the significant presence of Ova-specific Treg cells. Ultimately, it resolved spontaneously despite continued antigen expression in the skin. Rosenblum showed that when this engineered self-antigen was turned on in the skin, Treg cells there became activated, proliferated, and differentiated into more potent suppressors that mediated the resolution of the autoimmune activity. Then these activated Treg cells remained primed to attenuate subsequent autoimmune reactions when Ova was turned on again.

Rosenblum realized that tissues that have undergone autoimmune inflammatory reactions develop a property that serves to limit the severity of future such reactions. He called it *regulatory memory*, and the cells enabling it are the Tregs that survive after the initial encounter. They not only possess memory of the self-antigen—and thus are called memory regulatory T cells (*mTregs*)—but they have acquired an increased ability to suppress inflammation if and when this antigen reappears.

As with the more familiar T cells, the Treg's life history begins with generation in the thymus, then activation in the periphery, proliferation and differentiation into functionally more active cells, and then survival as memory populations.

Parsing the Biology of mTregs

Once Rosenblum realized that the skin-based encounter between Tregs and a self-antigen generates a population of skin-resident mTregs that remains in the skin just in case this antigen reappears, he wanted to identify the factors enabling them to stay.

Tregs carry receptors for IL-2 and some have the receptor for IL-7. When it comes to Tregs that develop in the thymus and return to secondary lymphoid tissues, both cytokines are involved in their development from naïve $CD4^+$ T cells and then in their maintenance. But when all of the action takes place in the skin, the roles for these two cytokines are more tighty scripted. IL-2 is essential for generating Tregs from their peripheral naïve $CD4^+$ T cell precursors. Then IL-7 acts independently to maintain some mTreg populations in the skin.

Rosenblum also learned that once the antigen is turned off, and thus no immune regulatory action is required, the mTregs localize preferentially to hair follicles—specifically, in the dermis surrounding the lower hair follicle segments. Later, this observation would turn out to be the first step in a momentous discovery.

Where Do Skin Tregs Really Come From?

Next, Rosenblum wanted to determine whether the antigen-specific Tregs that appear in the skin are derived in the thymus and then migrate to where they are needed, or whether the entire process can take place within the skin. He continued working with Ova rigged as a self-antigen, but instead of enabling his mice to produce their own T-cell-mediated response as he had done before, he transferred existing Ova-positive effector T cells (Teffs) from mice that had been engineered to produce T cells with a distinctive molecular signature. This way, any Treg cells derived from them would be easily distinguishable from immune cells produced by the host mice. So once the Ova-induced inflammatory response began and signaled the need for a regulatory counterbalance, if Treg cells appearing on the scene carried the host's molecular signature, then the track was thymus to skin. But if they bore the donor mouse signature, they clearly had evolved on-site, in the skin, from the donor's effector T cells. This time, adding or stopping doxycycline in the host mouse food turned Ova's presence on or off, and enabled Rosenblum to track events in the host mouse skin when these donor Teff cells first appear, the time until resolution, and what occurs when Ova is transient or persistent.

The Ova-specific donor Teffs induced an inflammatory dermatitis within 2 weeks after transfer, then stopped producing inflammatory cytokines after 2 weeks. Ova remained turned on. The inflammatory disease continued, fatal to 30% of the mice, and then resolved spontaneously after 40 days despite the continued presence of Ova and the donor

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Please address correspondence to:
Editors-in-Chief
Dermatology Focus
c/o The Dermatology Foundation
1560 Sherman Avenue
Evanston, Illinois 60201
Tel: 847-328-2256 Fax: 847-328-0509
e-mail: dfgen@dermatologyfoundation.org

Published for the Dermatology Foundation by
Robert B. Goetz
Designer, Production
Sheila Sperber Haas, PhD
Managing Editor, Writer

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DF Honors Excellence in Dermatology

The Dermatology Foundation pays annual tribute to dermatologists whose exemplary capabilities and dedication have helped to make the specialty what it is today. Presentation of the 2017 awards will be a highlight of the DF Annual Meeting on Saturday, February 17 in San Diego, CA. The leaders and role models being honored by their peers are:

Clark W. Finnerud Award—Paul I. Schneiderman, MD

Discovery Award—Luis A. Diaz, MD, and John R. Stanley, MD

Lifetime Career Educator Award—Ilona J. Frieden, MD

Practitioner of the Year—Lisa A. Garner, MD

(Drs. Diaz, Garner, and Stanley will be highlighted in the Spring issue.)

2017 Clark W. Finnerud Award: Paul I. Schneiderman, MD

Honoring the exceptional clinician who is simultaneously a dedicated and highly effective part-time teacher.

Dr. Schneiderman has maintained a bustling and varied medical dermatology practice in Syosset, Long Island, outside of Manhattan, for 40 years and it continues to excite him. "I relish receiving patients who are more complex, more challenging," he says. Known for his work with complicated patients, Dr. Schneiderman often receives referrals from internists and other dermatologists and consults for several nearby hospitals. He gives each patient "time, and my best intellectual effort. The greatest gratification is finding the problem and fixing it," he says. But this is just one facet of what he does.

During most of his career, Dr. Schneiderman has also been a part-time teacher. For more than 40 years, he has taught a weekly session at Columbia University Medical Center, using his extensive collection of kodachromes to challenge the residents on differential diagnoses, then teaches at the bedside during inpatient rounds. For 31 years, Dr. Schneiderman has held monthly educational sessions at Yale University. For the last ten years, he has also traveled on a quarterly basis to teach the residents at the University of Virginia (UVA), where he completed his internal medicine and dermatology residencies. A former student says "he was critical to the foundation of our clinical expertise. His mentorship is authentic and deep-seated."

Dr. Schneiderman came to dermatology via an atypical route. "I didn't pursue dermatology," he recalls. "It was dermatologists who pursued me!" Two chance encounters developed into lasting relationships with "a profound impact" on his career. During his medical residency at UVA, Dr. Schneiderman happened to meet second-year dermatology resident Dr. Ken Greer, who invited him to look at patients with him. "He piqued my interest and curiosity." After his medical residency, Dr. Schneiderman worked for the Public Health Service, then at the NIH, before beginning his dermatology residency at UVA. "On my first day at the NIH I met Dr. Rick Edelson, who asked if I was free to work in his lab with him." After Dr. Schneiderman returned to New York, Dr. Edelson invited him to teach weekly at Columbia, and later on a monthly basis at Yale.

Dr. Schneiderman is also an author. He and Dr. Marc Grossman coauthored the seminal *A Clinician's Guide to Differential Diagnosis in Dermatology*, with the second edition about to appear.

A colleague describes Dr. Schneiderman's contributions to the specialty. For decades, "he has graced his work as a teacher and a physician with his exceptional generosity of time, talent, and resources." Dr. Schneiderman views himself as a "very lucky person. I have worked with the smartest and most wonderful physicians. And I'm all the better for it."

DF Honors Excellence in Dermatology

2017 Lifetime Career Educator Award:
Ilona J. Frieden, MD

Recognizing an academic dermatologist with a career-long history of dedicated service as a mentor, role model, and inspirational teacher.

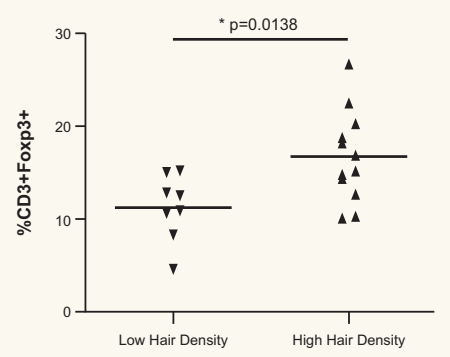
"I *love* pediatric dermatology!" exclaims Dr. Frieden, a world-renowned specialist in children's skin diseases with a substantial role in putting this subspecialty on the map. She is professor of Pediatrics and Dermatology at the University of California, San Francisco (UCSF), where she completed her pediatrics and dermatology residencies, and is now vice-chair of Dermatology and chief of the Division of Pediatric Dermatology. She founded a multidisciplinary Vascular Anomalies Clinic at UCSF in 1991. A decade later she founded the Hemangioma Investigator Group, a seminally important international research consortium.

While her pediatric training led to an interest in pediatric dermatology, Dr. Frieden's passion for her subspecialty was further ignited in 1983 during a 3-month Pediatric Dermatology elective with Dr. Nancy Esterly—one of the founders of pediatric dermatology and arguably the founder of academic pediatric dermatology—at Children's Memorial Hospital in Chicago. She also found Dr. Esterly to be truly inspirational—an excellent physician, a humble and curious person, and a wonderful role model, particularly for women entering the field. "It was the transformational moment in my career, and why I am who I am today," Dr. Frieden states. "Nan lit a fire within me, and helped me find my voice as a physician."

Dr. Frieden's career-long dedication to teaching and mentoring is well documented. She has served as a medical student and resident advisor, established the pediatric dermatology fellowship program at UCSF (in 2000), and has helped direct the dermatology department's Junior Faculty Mentoring Program. She joined the Mentoring Committee of the Women's Dermatologic Society at its inception in 1993 and received its *Mentor of the Year* award in 2005. When the AAD's Academic Dermatology Leadership Program (ADLP) was established in 2005, she became a mentor in that program, helping to provide long-distance mentoring for early-career academic dermatologists. In 2012, Dr. Frieden helped found the Pediatric Dermatology Research Alliance (PeDRA) to foster collaborative research, and serves as a young-investigator mentor in their mentoring program.

Dr. Frieden has also published extensively, held significant elected positions in specialty organizations, and is ending a long tenure as co-editor of *Pediatric Dermatology*. She co-authored the authoritative textbook in neonatal dermatology.

Dr. Frieden's influence on those entering her subspecialty has become legendary. One of her mentees says: "I could not have imagined the tremendous impact this would have on me. I was inspired by Dr. Frieden and her work. She helped me crystallize my goals and opened my world in ways I would never have expected." Another mentee explains, **"I spent a month with Dr. Frieden during my residency to expand my knowledge and experience with children. That month changed the course of my career. Two years later I returned to do a pediatric dermatology fellowship at UCSF—and have been here ever since!"**



Low hair density represents skin harvested from human anatomical sites with relatively lower hair follicle density (trunk, upper proximal extremities); *high hair density* represents skin harvested from higher hair density sites (scalp, face). (Reprinted with permission from *J Clin Invest.* See *Suggested Readings* for citation.)

T cells targeting it. Resolution reflected the generation and progressive expansion of Foxp3+ Tregs derived from donor T cells, a process anchored entirely within the skin. The abiding convention that Tregs were a relatively homogeneous population generated exclusively in the thymus and migrating to peripheral areas of need was now history.

Then Rosenblum and his team wanted to see if the duration of antigen exposure influences the final Teff/Treg balance when the mice are examined 60–80 days after receiving the donor's Ova-specific Teff cells. Turning Ova off after just 7 days left Teffs in the majority. But after persistent exposure—leaving Ova on for 60 days—Tregs dominated at ~80% of the mix. The Treg numbers were actually the same in both situations, but with persistent antigen exposure, the Teff population had plummeted.

"These studies highlight the essential role of peripherally generated Tregs in regulating local pathologic immune responses," Rosenblum points out. "They also emphasize the ability of Tregs to maintain their function in the face of constant encounter with a self-antigen, and this may lead us to a better understanding of the pathogenesis of autoimmune disease. Elucidating the critical pathways that control the Teff/Treg balance may provide insight into the pathogenesis of tissue-specific autoimmunity."

Tregs in Human Skin—The First Assessment

Now it was time to see if the observations in mouse skin held true for human skin as well. Before Rosenblum and his team could begin their assessment, though, they had to devise a way to digest skin samples gently enough to enable isolation of a suffcient number of undamaged leukocytes for study, eliminating the need for growth factors and days of culture that distorted the cellular landscape. Applying their new method overnight to surgically discarded skin samples from healthy patients revealed that ~20% of the CD4+ T cells in adult skin express Foxp3—ie, they are Tregs. And the almost uniform presence of the memory marker CD45RO expressed by these Tregs meant they had previously seen antigen outside of the thymus—in peripheral tissue— consistent with a memory T cell phenotype. Rosenblum was able to demonstrate that these mTregs were activated memory cells, and that they do not migrate out of the skin.

In a particularly critical parallel, when these human mTregs were not involved in immunoregulatory activity, they preferentially localized to hair follicles in close proximity to the follicular epithelium. In line with this, flow cytometric quantification showed that skin with high hair density—the scalp and face—had significantly higher percentages of Tregs than areas of low hair density (see graph at left).

In total, this first informed exploration of mTregs in human skin documented a spectrum of distinctive identifying characteristics. This applied to cell surface marker expression, cytokine production, *in situ* localization, T-cell receptor, and functional capacity. These residents in human skin are clearly a unique subset of Tregs.

Rosenblum had access to punch biopsies from psoriasis patients with active disease, and wanted to see how chronically inflamed and nonlesional skin compared to skin from healthy individuals when it comes to immunoregulation. He and his team optimized their skin digestion protocol further to provide a sufficient number of cells from 4-mm punch biopsies. Compared to nonlesional skin, the mTregs in lesional skin were more numerous, highly proliferative, and produced low levels of IL-17. Their role in the disease process remains to be explored.

Multi-tasking Tregs

Both mouse and human skin contain a particularly large number of tissue-resident Tregs, which makes sense given that the skin is an organ of the immune system and our immediate interface with the environment. Given the broad scope of the skin's essential protective functions and the fact that this Treg population remains permanently in the skin, the possibility arises that these cells—so critical for immune homeostasis—may also have other important functions in the skin. Rosenblum found several possibilities particularly compelling for his initial explorations: ensuring effective wound healing (because of the shared involvement with the EGFR—epidermal growth factor receptor—pathway); control of hair follicle stem cell behavior (because the hair follicle is where Tregs cluster); and safeguarding the skin's commensal bacteria (essential to health, they need to be protected from immune attack).

Wound Healing

Because the skin is highly susceptible to traumatic injury, wound healing is a critical protective function. Wound healing is mediated by multiple cell types and molecular pathways, with the EGFR pathway—which stimulates both epidermal and dermal regeneration—playing a major role in both. Rosenblum noted an intriguing molecular intersection between wound healing and Tregs in the skin. AREG—the growth factor amphiregulin—is the EGFR ligand. It is also expressed by Tregs that reside in muscle and lung, and enhances repair in these tissues after injury.

Rosenblum considered two ways in which the skin's Tregs might be involved in wound healing support. "Because they play a major role in mediating skin immune homeostasis, we set out to determine whether they help to attenuate wound-associated inflammation," he says. "We also wanted to see if they use the EGFR pathway to facilitate wound repair itself." They found that in response to full-thickness wounding, highly activated Tregs expand in skin and play a major role in limiting production of the key macrophage activator IFN-γ, thus minimizing the accumulation of proinflammatory macrophages. In addition, removing Tregs from the wound-healing equation by ablating them early after wounding significantly delayed re-epithelialization and the kinetics of wound closure. (see photo on page 9). So Tregs turn out to be critical players in wound healing, limiting wound-associated inflammation on the one hand and directly enabling normal wound repair to progress on the other.

Rosenblum points out that some therapeutic approaches to treating chronic skin ulcers have focused on increasing EGFR signaling. "Our data suggest that these strategies may work, in part, by augmenting cutaneous Treg function," he reflects. And it may be inadequate or ineffective Tregs that set the stage for chronic ulcers in the first place. Rosenblum believes that "direct therapeutic manipulation of Tregs may be a novel strategy for treating wound-associated inflammation with the potential to expedite healing."

Regulating the Hair Follicle Cycle

The maintenance of tissue homeostasis is critically dependent on two elements—the competence of tissue-resident immune cells and the differentiation capacity of tissue-resident stem cells (SCs). The hair follicles and Tregs are so intimately intertwined that if hair follicle development is knocked out, Tregs do not migrate into the skin. Because the skin Tregs preferentially localize to hair follicles, which house a major subset of skin stem cells (HFSCs), Rosenblum wondered if they participate in some way in the differentiation of epithelial SCs.

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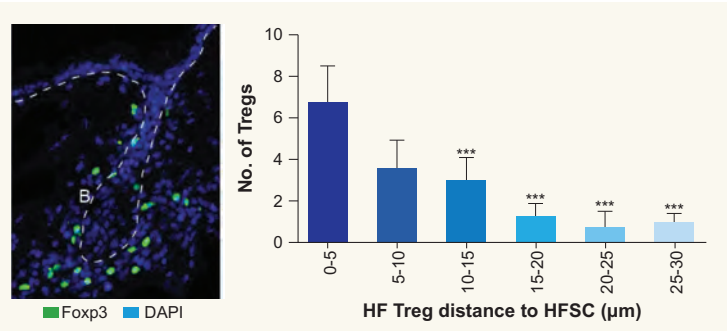


Tregs cluster around bulge. ***Left:*** Immunofluorescence microscopy shows Foxp3+ Tregs *(green)* clustering around the bulge region *("B")* of a mouse hair follicle in telogen skin. Dashed line outlines the hair follicle. (DAPI is a fluorescent blue stain.) ***Right:*** Quantification of fluorescent Foxp3+ Tregs within 30 μm of follicular epithelium shows majority residing within 0–5 μm of bulge HFSCs. ***p<0.001. (Reprinted with permission from *Cell*. See *Suggested Readings* for citation.)

Hair follicles are highly specialized organelles that are in a perpetual state of growth and regeneration. A major epithelial SC population localizes to hair follicles (along with the Tregs residing there), with dual responsibilities. They are indispensable to hair follicle regeneration and to repair of the epidermal barrier after injury. There were also some provocative data links between Tregs and hair follicle biology. Genome-wide association studies in alopecia areata (AA), a disorder of hair follicle regeneration, have revealed single nucleotide polymorphisms in genes involved in Treg differentiation and function. They include IL-2, the high-affinity IL-2 receptor alpha, CTLA-4, Eos, and Foxp3. In addition, using low-dose IL-2 for pharmacologic augmentation of Tregs in people with AA had been highly efficacious.

"Yet despite all of these associations," Rosenblum points out, "a functional link between Tregs and hair follicles had yet to be established." Knowing that Tregs in bone marrow co-localize with hematopoietic SCs—and had been found to support SC function in this tissue—amplified his curiosity further.

"We began by performing comprehensive immune profiling of Tregs in mouse skin at specific stages of the synchronous HF cycle," Rosenblum says. Looking first at skin-draining lymph nodes, they found that the number of Tregs showed little variability in number. But in the skin itself, accumulation was not only highly variable, this variance correlated tightly with HF cycling. Tregs were roughly 3 times more abundant in the telogen phase compared to anagen. After establishing that Tregs play a major role in facilitating hair follicle regeneration by promoting the telogen-to-anagen transition, Rosenblum looked more closely at the Tregs' location in the complex geography of the hair follicle where they reside. He wanted to see if they co-localize specifically with HFSCs themselves. Immunofluorescence microscopy enabled him and his team to document that they cluster around the bulge region of the lower portion of telogen hair follicles (see photo at left), which is a well-established niche for HFSCs. Specialized staining revealed a subpopulation of bulge-associated Tregs in close association with HFSCs themselves. And the majority of follicular Tregs locate within 0–5 μm of bulge HFSCs (see graph at left). Using intravital imaging for real-time observation of the behavior of Tregs closest and farthest from the follicular epithelium provided additional support. Tregs were highly active in the bulge region and their potential for communicating with bulge-associated HFSCs was apparent. (See photo on cover.) In another piece of the puzzle, lineage-specific depletion of these cells resulted in marked attenuation of hair follicle regeneration. Rosenblum and his team also discovered that—at least in mice—signaling through the Notch pathway is a major mechanism by which Tregs promote HFSC function. Rosenblum suspects that this relationship between Tregs and HFSC function exists in humans but that the relevant pathway will turn out to be different.

"Taken together, these results suggest that suppression of inflammation is *not* the major mechanism by which Tregs promote HFSC proliferation and differentiation," Rosenblum points out. "Our data suggest that Tregs in skin have the ability to modulate the biology of tissue SCs independently of this function, which is a fundamental departure from the way Tregs are thought to work, ie, the traditional view of regulating inflammation. Although the overwhelming body of literature suggests that this is the sole function of these cells," Rosenblum continues, "we and others have clearly shown that they act in important ways that are independent of their ability to suppress inflammation. And that is the major crux of this area of our research—that regulating inflammation is not the central mechanism mediating this effect of Tregs on hair follicles. It is a huge departure in the way that people think about these issues," Rosenblum concludes. "We are deviating from doctrine."

Tregs and Commensal Microbes

Our commensal microbiota are protective, residing primarily at barrier sites—eg, the gastrointestinal tract, respiratory tract, urogenital tract, and skin—where they functionally tune our innate and adaptive immune systems. Immune tolerance to these resident microbes has to be established at each barrier site, and until Rosenblum's lab began to study this phenomenon in the skin, research had focused almost exclusively in the gastrointestinal tract.

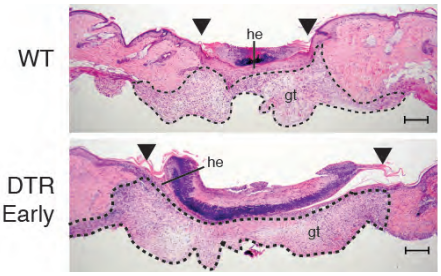
Yet the skin is a key barrier site and a rich immunologic organ. Each square centimeter contains over a million lymphocytes and a million commensal bacteria, pointing to a constant dialogue between the immune system and these microbial residents. And as our external body surface, the skin regularly sustains contact with a spectrum of exogenous microbes. The skin is also a far more complex tissue than the other barrier sites, a stratified and cornified epithelium with a diverse topography studded by adnexal structures that include hair follicles, sweat ducts, and sebaceous glands. The maintenance of a healthy immune dialogue with commensal microbes is not simple, and recent research suggests that disrupting the interactions between Tregs and skin commensals might have enduring health implications.

Rosenblum and his team—led by Tiffany C. Scharschmidt, MD, assistant professor in the department who now has her own lab—demonstrated that immune tolerance to commensal microbes is established during a precise period of neonatal life, a crucial window defined by an abrupt influx of highly activated Tregs into neonatal skin. And that is when the hair follicles—which are a primary reservoir for skin commensals—begin to poke through the skin and the commensal microbes descend. Tregs are recruited at this point to establish tolerance to the microbiota. Selective inhibition of this Treg wave completely abolishes commensal tolerance.

Moving on to identify what initiates this wave of Treg migration into neonatal skin, Rosenblum and his team found that the neonatal hair follicle and commensal microbes interact to increase expression of the chemokine Ccl20. The receptor that responds to Ccl20, called Ccr6, is preferentially expressed by Tregs in that early period. This hair follicle-microbiota teamwork recruits Treg cells into skin, which then function to protect these commensals from immune recognition.

Treating Autoimmune Disease—It's All About Balance

"We used to believe that people who develop autoimmune disease were unlucky, in that they had autoreactive immune cells that had escaped detection in the thymus or periphery and were now wreaking havoc in the tissue," Rosenblum notes. "But now we think that *everybody* has such cells. And the reason that most people do not go on to develop autoimmune disease is that their Treg cells are keeping their autoreactive immune cells in check." Autoimmune disease develops when Treg activity is deficient and this balance is no longer maintained. This would also explain why it is common for patients to have more than one autoimmune disease.



Tregs and wound healing. Histology at 7 days after wounding compares the rapid healing of wild-type mice *(WT)* with the slow healing of mice whose Tregs were silenced after wounding *(DTR Early)*. Arrowheads denote wound edges. (Reprinted with permission from *J Immunol. Copyright 2016. The American Association of Immunologists, Inc.* See *Suggested Readings* for citation.)

This new understanding dramatically redefines—and simplifies—the therapeutic target. It will no longer be eliminating the specific population of autoreactive immune cells for an individual autoimmune disease. Instead the goal will simply to augment the Treg population and restore the body's natural ability to regulate this. It is truly a one-size-fits-all treatment for autoimmune disease, because it is approaching the basic cause, not a symptom of that cause. Giving a pharmacological agent that augments the immune regulatory presence to dampen the pathologic effector response will reset the balance of the immune system. "I believe that this is the future of treating autoimmune diseases," Rosenblum states.

Final Thoughts

"One of the biggest surprises we have had so far is how many diverse functions Tregs have in the skin," Rosenblum emphasizes. "The skin is home to a large portion of the body's Tregs, which speaks to the fact that these cells are definitely a major player in this tissue. And we are beginning to understand that they have a diverse array of functions within this tissue, related to context. Traditionally we think of these cells as defined within the narrow perspective of conventional immune responses. But in the tissue, they do that—and so much more! And it's the 'so much more' that's been really surprising."

Rosenblum continues to probe this "so much more." He has been looking more closely at the contribution of skin-resident Tregs to the maintenance of barrier function and the restoration of barrier tissue integrity after injury, and a possible interaction with fibroblast function that may have implications for understanding and treating fibrotic skin disease. Stay tuned.

Suggested Readings

Ali N, Rosenblum MD. "Regulatory T cells in skin." *Immunol.* 2017;152:372–81.

Rosenblum MD, Way SS, Abbas AK. "Regulatory T cell memory." *Nat Rev Immunol.* 2016;16:90–101.

Rodriguez RS, Pauli ML, Heuhaus IM, Yu SS, et al. "Memory regulatory T cells reside in human skin." *J Clin Invest.* 2014;124:1027–36.

Nosbaum A, Prevel N, Truong HA, Mehta P, et al. "Cutting Edge: Regulatory T cells facilitate cutaneous wound healing." *J Immunol.* 2016;196:2010–4.

Ali N, Zirak B, Rodriguez RS, Pauli ML, et al. "Regulatory T cell in skin facilitate epithelial stem cell differentiation." *Cell.* 2017;169:119–29.

Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, et al. "A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes." *Immunity.* 2015; 43:1011–21. ■

Dr. Rosenblum received funding from the Dermatology Foundation: 2009 Investigator Research Fellowship; 2010 Career Development Award; 2015 Stiefel Scholar Award.

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Focus on Research

Adipose Stem Cells—Understanding Them and Their Potential Value for Aesthetic and Regenerative Medicine

(Continued from cover)

handling or culture, and they stimulate a variety of helpful growth and healing pathways without raising the risk of cancer or initiating a significant immune response.

Thus autologous adipose tissue has become the go-to approach for a disparate variety of problems, a number of which are also commonly treated by dermatologic surgeons. Percec relies on it for restoring facial volume lost with aging, and for treating soft tissue volume loss acquired through trauma or congenital malformation. She uses it for augmenting the buttocks and breasts, for restoring breasts after lumpectomy, and for reconstructing them after mastectomy. She uses autologous fat to normalize fibrotic skin after radiation damage. She relies on it to help sluggish wounds heal. And it is critical for female genital reconstruction after ritual mutilation. But that is only part of the story.

Critical Questions Remain Unanswered

The type of research needed to support optimal use of autologous fat grafts has not kept pace, which has generated Percec's deep concern. She understands that the true potential of autologous fat grafting cannot be adequately recognized or accessed until this research is done—and done properly.

On the one hand, there are no reliable methodologic guidelines for maximizing the probability of a successful outcome. "We do not know how to harvest the tissue or inject the lipoaspirate properly, or optimize our results—so only about 40–60% of what we inject remains," she says. Disagreement continues even on the most effective tools to use throughout the process.

And there are critical unanswered questions on the role of ASCs in successful graft survival, despite the recognition that human adipose tissue is the ideal mesenchymal stem cell source for the adult stem cell-based therapies that have become a primary focus in regenerative medicine. The appeal of ASCs reflects their compelling profile (see box on page 12), including their outstanding multipotentiality. Roughly 130 active clinical trials are listed by the NIH. Yet the mechanisms regulating these cells before and after harvest, as well as their behavior after *in vivo* transplantation—differentiation, migration, and cell survival—remain to be identified and understood. And the basic issues of ASC preservation have yet to be assessed. This range of concerns regarding the current use of autologous fat grafts and the hoped-for benefits of ASCs is shared by dermatologists involved in aesthetic and reconstructive surgery.

Goals

This is the territory that Percec is exploring in her lab, using human adipose tissue and working at the molecular level. She believes it is crucial to move away from *in vitro* preparations and animal models and focus on human cells, because roughly 90% of the discoveries made in mice or other animal models do not translate to humans. Although the genetic heterogeneity among people makes human cells more difficult to work with than cells from a controlled animal population with a single genetic makeup, Percec knew that the direct relevance of data would be worth the challenge, and deriving stem cells directly from her patients would give her complete control over all of the clinical variables and storage aspects. After working out the antecedents, she has launched her pursuit of the complex regulatory mechanisms controlling adipose tissue and ASC biology—*in vivo*, in storage, and after transplant—to identify the molecular pathways that will optimize the function of these stem cells.

Adipose Stem Cells—A Model for Soft Tissue Aging

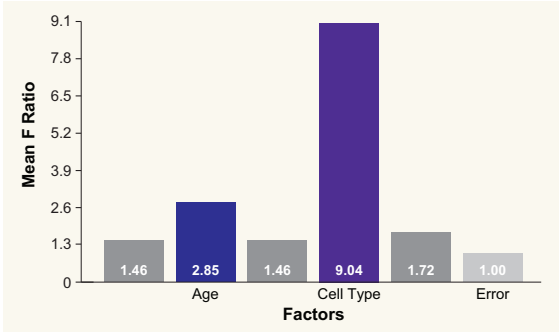
A primary goal of Percec's has been to use these stem cells as a relevant model for human soft tissue aging that would allow her to study the process and evaluate potential interventions. The mechanisms responsible for soft tissue aging are becoming progressively more important as the world's population ages, yet most studies on aging have been conducted either in animal models—typically mice—or with an artificial model such as cells cultured from progeria patients, or cells in the culture flask forced to replicate to senescence. Because none of these represents normal human aging, clearly relevant molecular pathways and alterations had yet to be identified. Percec was convinced that human adipose tissue from healthy individuals can generate this knowledge, and that what she would learn as she studied them should ultimately also help to understand—and hopefully improve—the clinical experience with autologous fat grafting. Advanced patient age reduces graft success as well as ASC quantity and viability; fat from different anatomic compartments may age differently; fat from men and women may have significant aging differences.

To prepare for this study, Percec conceived of a preservative-free method for freezing freshly harvested adipose tissue. The absence of preservatives enables her to maintain the stem cells as close to their natural state as possible and avoid distorting or impairing their function. After perfecting the approach, then building her tissue bank, Percec looked for differences in genomic transcriptional profiling—ie, RNA profiles that indicate the individual pattern of gene expression—between the primary cell types in adipose tissue, and between youthful and older age groups.

Percec studied subcutaneous adipose tissue that she had excised from the anterior abdomen of 6 healthy patients—3 young (26–29 years) and 3 older (52–64 years)—during cosmetic procedures. She isolated adipocytes and stromal vascular fractions for genome-wide transcriptional analysis, looking for gene expression differences between cell types and between age groups. She found them. Age showed an increase in overall gene expression among older patients but, very unexpectedly, these differences were extremely modest. Pronounced differences were identified between cell types. Age-related differences were a very distant second. (See bar graph below.)

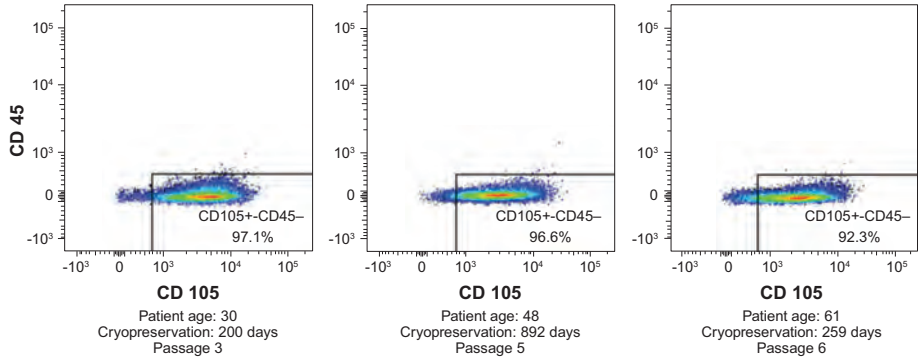
Long-Term Storage and Age: Exploring the Impact

Thinking ahead to potential future acceptance of ASCs in regenerative applications, Percec knew that "advancing patient age was believed to correlate with impaired ASC differentiation and growth profiles." The implication was that for the large majority of patients, by the time emerging health issues require regenerative treatment, a patient's freshly harvested ASCs would no longer be functioning optimally. Perec's envisioned



Microarray analysis to identify differentially expressed genes profiled freshly harvested adipocytes and stromal vascular fraction components from 3 young (26–39 years) and 3 old (52–64 years) healthy patients. Cell type was the most significant contribution to transcriptional variation; age was a distant second. (Reprinted with permission from *Ann Plastic Surg.* See *Suggested Readings* for citation.)

solution is a standard practice of banking subcutaneous adipose tissue while a person is young and healthy, then years later—if and when needed—retrieving their cryopreserved tissue to isolate and use stem cells. Contrary to the approach for creating stem cell reserves from umbilical cord blood—isolating, expanding, and storing only the stem cells—Percec believes in storing the entire tissue to keep the stem cells within their nourishing and protective niche until they are needed. With this in mind, she looked at the effect of cryopreservation length and patient age on ASCs freshly harvested from cryopreserved adipose tissue.



Sorting ASCs by flow cytometry to characterize the molecular marker profile for cells from adipose tissue harvested from patients of different ages and after different cryopreservation durations showed the retention of both stemness and proliferative ability in the great majority of stem cells from each source. (Reprinted with permission from *Stem Cells Int.* See *Suggested Readings* for the citation.)

The Biology and Benefits of Subcutaneous Adipose Tissue

Human fat—a mesenchymal tissue and the largest contributor by volume to the connective tissue matrix—is composed of matrices of adipocytes interspersed with collagen fibers and the stromal vascular fraction (SVF). The SVF includes preadipocytes (adipose precursor cells), adipose stem cells (ASCs), fibroblasts, pericytes, vascular endothelial cells, and immune cells (tissue macrophages, B cells, and T cells). The first hint that human adipose tissue is not simply an inert filler that stores lipids, cushions our organs, and helps to keep us warm came just over 20 years ago. In 1994, the first adipokine—a cytokine secreted by adipose tissue—was discovered, with hundreds more to follow. As recently as 2010, scientists identified an additional 20 proteins not previously detected in human fat cells, and 6 novel proteins never seen before.

Adipose tissue is collectively the body's largest endocrine organ, with an exceedingly complex and varied profile. It produces and releases a vast array of protein signals that include growth factors, cytokines (including TNF and IL-6), chemokines, acute-phase proteins (which increase or decrease in response to inflammation), complement-like factors, adhesion molecules, components of the extracellular matrix, and hormones. These trophic factors substantially enhance ASC impact. Changes in adipose tissue function can be triggered by either plasma membrane receptors (for insulin, glucagon, growth hormone, adiponectin, gastrin, and angiotensin-II) or nuclear receptors (for PPAR , estrogens, androgens, vitamin D, thyroid hormone, progesterone, and glucocorticoids). Thus adipose tissue is vital to metabolic homeostasis, immune regulation, angiogenesis and coagulation, and contributes significantly to regulating reproduction, fibrinolysis, vascular tone control, and body weight homeostasis. It is also a factor in longevity.

Of special significance is that subcutaneous adipose fat—85% of all adipose tissue in the human body—is the largest known reservoir of adult stem cells, potentially the most abundant source of regenerative cells in the human body. Their immunogenicity is very low, and ASCs can differentiate into a substantial range of cell types including osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic cells, and hepatocytes. ASCs' strong paracrine signaling mechanisms confer multiple protective effects. They are anti-inflammatory, proangiogenic, immunohomeostatic, protect against neurodegeneration and cancer, and promote scarless wound healing. Their secretion of cytokines, chemokines, and growth factors stimulates the body's innate regenerative capacity in a wide range of applications, and the trophic factors expressed by the tissue enhance this.

She and her team used tissue from patients 26–62 years old that was excised during abdominoplasties and then cryopreserved from 2–1,159 days (slightly >3 years). ASC number and viability were assessed at the initial excision, then measured post-harvest—along with rate of growth—after 9, 18, and 28 days of growth.

Although significantly more viable cells were initially isolated from tissue cryopreserved for less than 1 year compared to cryopreservation beyond 2 years, this difference disappeared as the growth phase continued. There were no significant differences in stem cell viability or growth rate at subsequent time points regardless of cryopreservation duration or patient age. So although longer cryopreservation negatively affects the initial live ASC isolation results, this effect is neutralized as the growth phase continues. As for the effect of patient age, comparing cells from patients <40 years with patients ≥50 showed no significant differences in initial ASC viability or in growth after cryopreservation (see graph on page 14). Mesenchymal stem cell markers were maintained in all age cohorts throughout the study's duration (see graphs at left). And ASCs from all subgroups were equally capable of undergoing both osteogenic and adipogenetic differentiation.

Percec and her team were so unprepared for the capacity of these adipose-derived stem cells to preserve their youthfulness "that it took several years for us to accept these findings and stop wondering if our models were not adequate or we were not including a sufficient number of patients," she comments. "But it became clear that the older stem cells could not only be harvested after years of being stored, they could replicate almost as well as younger stem cells. They maintain their stability—although we do not yet fully understand how."

The maximum duration of cryopreservation is currently 7 years now, and harvesting the original samples continues to produce reliably effective stem cells. "This study provides evidence that whole adipose tissue cryopreservation can serve as the gold standard for long-term ASC biobanking," Percec says. "Taken together, our data suggest that ASCs preserved within their tissue niche are capable of enduring long-term cryopreservation while maintaining their stemness. Whole-tissue cryopreservation avoids any potential adverse effects caused by cellular trauma or by the use of cryopreservative media," she points out. "Because of the incremental decrease in subcutaneous adipose tissue mass with advancing patient age," Percec

(Continued on page 14)

New 2017 *Leaders Society* Members Invest in Progress

The Foundation welcomes the forward-looking dermatologists who chose to join their colleagues in the Leaders Society, and appreciates their confidence in the DF's ability to identify and support tomorrow's leaders driving progress in the specialty. Their wise decision to invest $1,500 annually in this effort will translate to advancing patient care for years to come.

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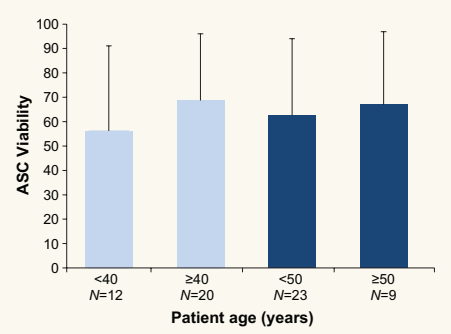

ASC viability was compared between the following age cohorts: <40 vs ≥40, <50 vs ≥50, and <40 vs ≥50. No significant differences were observed. (Reprinted with permission from *Stem Cells Int.* See *Suggested Readings* for the citation.)

continues, "it behooves us, when feasible, to bank this valuable tissue prior to its diminution, ideally by middle age."

Digging Deeper

To begin exploring what underlies the human ASC's remarkable stability during the progression to early aging, Percec and her team decided to compare ASCs from healthy women between ages 24–64 with two other cell populations. One comprised freshly harvested age-matched dermal fibroblasts. The other—a common model of cellular aging called IMR-90—is a human fetal lung fibroblast pushed to senescence by forcing an extensive number of cell doublings. Percec divided the stem cell and fibroblast populations into younger and older subgroups, then assayed the transcriptome of each subgroup and the IMR-90 cells. This basically produced an RNA map for each group showing the genes being actively transcribed, and each gene's relative share of the RNA pool. Percec and her team were looking for genes associated with age-related regulation that departed significantly from the youthful pattern with a more than twofold increase or decrease in expression.

The differences were clear and dramatic. Age-related gene expression changes were minimal in the ASCs, compared to dramatic age-related changes in the other two cell groups. In early-aging ASCs, only 279 genes were strongly upregulated and 95 were downregulated. But these numbers were far greater in aging fibroblasts and senescent IMR-90 cells—1,180 and 857, and 660 and 1,778, respectively. The effect of the altered gene expression in aging ASCs basically speeds up gene translation and the cell cycle. Just the opposite occurs in the other two cell groups, where these processes slow down considerably. "These synergistic modifications suggest that multiple regulatory events at the transcriptional, translational, and post-translational levels act together to enable aging ASCs to maintain their 'stemness' and tissue homeostasis," Percec explains. "Our results reveal novel chronological aging mechanisms in ASCs that are inherently different from differentiated cells," she continues, "and that may reflect an organismal attempt to meet the increased demands of tissue and organ homeostasis during aging." These robust cells do the work needed to retain stable youthful function.

In Progress

The SIRT1 enzyme is critical to cellular health and longevity. Percec is assessing its role in healthy ASC function, and possible deficiency in impaired ASCs—eg, from aged or obese patients—that can no longer produce sufficient adipocytes and are typical of defective autologous grafts. She will also see if deficient status can be reversed. A related issue is the role of mitochondrial activity in healthy SIRT1 function, which Percec is also exploring in the context of healthy vs defective ASCs. Again, pharmacological reversal of any identified mitochondrial deficiency will be attempted. Yet another focus targets the potential for improved wound healing. Percec will induce ASCs from healthy female patients (ages 25–59 years) to differentiate into fibroblasts, and their ability to produce extracellular matrix and generate rapid healing with less scarring will be compared to dermal fibroblasts harvested from these same patients. In another vein, Percec is intrigued with the juxtaposition of normally mutually exclusive characteristics in ASCs. On the one hand these stem cells are exceptionally quiescent, which protects them from the oncogenic potential that is a disadvantage of so many other stem cells. But these same stem cells also show remarkable regenerative capacity. "This is one of the puzzles we are trying to decipher," Percec says. And last but not least, they are going to evaluate the ASCs from an obese population to see if they respond differently to aging and to cryopreservation.

Thoughts About the Future

Percec covers both ends of the time spectrum when she comments about her hopes for progress in using adipose tissue and ASCs therapeutically. Concerning the current clinical applications of autologous fat grafts, "we are still so far behind in developing the ideal methodology for treating fat properly and allowing it to have a much more robust and predictable viability once it is transferred," she cautions. And thus Percec looks forward to the research that is urgently needed to establish the most effective methodology. And looking to the future, Percec points out that ASCs have even shown therapeutic benefit in animal models of cardiac ischemia or stroke. "And when they are injected directly into the animal's bloodstream, they actually target the injured tissue," she points out. "Can this be our goal down the line? It would be amazing!"

A Timeline of Firsts

1893: The German plastic surgeon Gustav Neuber discussed his novel approach—autologous fat grafting—to filling a soft tissue facial defect (a large conical infraorbital scar from childhood tuberculous osteitis) in a young male patient, and satisfying cosmetic outcome.
1895: The first breast reconstruction took place, by transferring a lipoma from the patient's buttock.
1910: The first report appeared of fat injection by a plastic surgeon.
1920: The first book appeared devoted completely to fat grafting.
1950: The first studies appeared on the biology of fat graft survival.
1980: Liposuction was developed, greatly facilitating fat harvest.
1987: The first experiences appeared using liposuctioned fat for breast augmentation, but issues in demonstrating efficacy and safety delayed approval by the American Society of Plastic Surgeons for almost 20 years.
2001: Stem cells were identified in human adipose tissue at UCLA's Laboratory for Regenerative Engineering and Repair.
2007: The first report appeared on the benefit of fat grafting for treating radiation-induced tissue damage.

Suggested Readings

Hsu VM, Stransky CA, Bucky LP, Percec I. "Fat grafting's past, present., and future: why adipose tissue is emerging as a critical link to the advancement of regenerative medicine." *Aesth Surg J.* 2012;32:892–9.

Stransky CA, Hsu VM, Dierov R, Hoover WJ, et al. "Beyond fat grafting: what adipose tissue can teach us about the molecular mechanisms of human aging." *Ann Plastic Surg.* 2012;69:489–92.

Devitt SM, Carter CM, Dierov R, Weiss S, et al. "Successful isolation of viable adipose-derived stem cells from human adipose tissue subject to long-term cryopreservation: positive implications for adult stem cell-based therapeutics in patients of advanced age." *Stem Cells Int.* 2015;2015:doi: 10.1155/2015/146421.

Shan X, Roberts C, Kim EJ, Brenner A, et al. "Transcriptional and cell cycle alterations mark aging of primary human adipose-derived stem cells." *Stem Cells.* 2017;35:1392–1401.

Gersch RP, Glahn J, Tecce MG, Wilson AJ, et al. "Platelet rich plasma augments adipose-derived stem cell growth and differentiation." *Aesth Surg J.* 2017;37:723–9. ■

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"The DF is critical to the future of dermatology."

Giving Back—The New Chair of the Annenberg Circle Committee

Dermatology has matured into an important specialty within the house of medicine. Continued progress is enabled by dedicated volunteers like Dr. Elizabeth McBurney, helping the DF increase its support of the work that will drive advancements in patient care. Adding to a devoted volunteer history that began in 2000, Dr. McBurney has just taken on the role of Chair of the Annenberg Circle Committee from departing Chair, James O. Ertle, MD.

Dr. McBurney is highly enthusiastic about this new opportunity to help the specialty by bringing her talents to growing AC membership. **"The DF is absolutely critical to the specialty," she points out. "It was designed by dermatologists, for dermatologists, specifically to help develop advance knowledge and patient care. AC contributions enable the Foundation—and the essential support it provides—to continue long into the future."**

Elizabeth I. McBurney, MD

Dr. McBurney is clinical professor of dermatology at both Louisiana State University School of Medicine and Tulane University School of Medicine in New Orleans, and is especially interested in T-cell lymphoma, laser surgery, and skin rejuvenation. She became a *Leaders Society* member in 1992, and since then has significantly increased her support of the DF as a member and a volunteer, including service as Secretary-Treasurer.

Dr. McBurney worked with Dr. Ertle as vice chair of the AC Committee since his chairmanship began in 2013. "He has given so unselfishly to the DF of his time and dollars over the years," Dr. McBurney notes. "His unflagging enthusiasm for the Foundation serves as a beacon for the rest of us. If I am as successful as he was, I will be very grateful," she adds.

Dr. McBurney is extremely pleased to welcome Kishwer S. Nehal, MD, to the committee as vice chair. Dr. Nehal is director of Mohs and Dermatologic Surgery at Memorial Sloan-Kettering Cancer Center in NYC. "With her boundless and infectious energy, she will be a real asset to the committee," Dr. McBurney says.

The DF is exceptionally grateful to its many volunteers who give so generously of their time to keep dermatology at the forefront of medicine.

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