Predicting allograft tolerance

By Lev Osherovich, Senior Writer

Based on an analysis of two groups of kidney transplant patients able to thrive without immunosuppressants, consortia in Europe and the U.S. have independently identified immature B cells as markers that could help pinpoint transplant patients who may not need immunosuppressants. Miltenyi Biotec GmbH and Pangaea Biotech S.A. have options to develop diagnostics based on the discoveries, which were reported in back-to-back papers in *The Journal of Clinical Investigation*.^{1,2}

The consortia—the EU-based **Indices of Tolerance**, the EU-based **Reprogramming the Immune System for the Establishment of Tolerance** and the U.S.-based **Immune Tolerance Network**—were searching for clues about why some patients don't reject transplants.

Patients who are naturally tolerant of allografts are hard to identify because long-term immunosuppressive therapy is the standard of care after transplant surgery. However, some patients stop taking the drugs because of side effects, toxic cross-interactions with certain cancer therapies or outright noncompliance. Among these patients, most undergo immunological destruction of their donated organs. However, a small fraction appear to suffer no ill consequences and retain their grafts.

Although there likely are allograft-tolerant patients who remain on immunosuppressive therapy, there is currently no way to identify them.

"Most patients who stop taking immunosuppressants will reject the graft, but we have no idea how many patients could stop taking the drugs and get away with it," said Maria Hernandez-Fuentes, senior fellow of translational research at **King's College London**.

Hernandez-Fuentes is the corresponding author of the report by the two European consortia and a coauthor of the American study.

"We're looking for good tests that provide mechanistic explanations" for graft tolerance, said Laurence Turka, co-director of the Division of Transplant Immunology in the Department of Medicine at **Beth Israel Deaconess Medical Center** and **Harvard Medical School**. "The first step is to understand what tolerance looks like."

Turka is deputy director of tolerance assays and data analysis at the Immune Tolerance Network and a corresponding author on the American group's study.

Foot in the door

To get a grip on allograft tolerance, the teams collectively studied the immune cell composition and gene expression profiles of 30 kidney transplant patients who had stopped taking immunosuppressants for at least 1 year with no adverse effect. The European collaborators studied 11 patients, whereas the Americans studied 19.

Both studies found that allograft-tolerant patients had higher levels of circulating B cells compared with control patients who remained on immunosuppressive therapy. Despite the higher B cell counts, the tolerant patients generally did not have antibodies against their grafts.

A clue about why these excess B cells did not make antibodies came from gene expression experiments. Compared with blood samples from healthy or immunosuppressed controls, those from tolerant patients had higher levels of genes associated with immature B cells that do not yet produce antibodies.

Hernandez-Fuentes said that the potential role of B cells came as a surprise. Previously, allograft tolerance was viewed as a consequence of increased numbers of T_{reg} cells, which inhibit immune reactions. However, the European team found no differences in T_{reg} cell levels or activity between tolerant and control patients.

The question now is whether the altered B cell counts are a cause or consequence of allograft tolerance.

"Because these people have been off of immunosuppression for so long, it's not clear whether the mechanism that allows this involves the B cells or whether it's something earlier," said Vicki Seyfert-Margolis, a coauthor of the U.S. study. Seyfert-Margolis is now senior advisor on science innovation and policy in the FDA's Office of the Commissioner.

Both teams looked for signs that B cells directly contributed to tolerance but got conflicting results. The American group found that immature B cells isolated from tolerant patients produced more IL-10, an anti-inflammatory cytokine, than B cells from controls.

However, the European team found no difference in IL-10 levels between tolerant and control groups.

Colin Macaulay, director of R&D at **Viron Therapeutics Inc.**, echoed Seyfert-Margolis' question. He said that although "human studies have shown that a robust antibody response is linked to poor outcomes" such as graft rejection, the "jury is still out" on whether the altered B cell profile is a cause of tolerance or a consequence of earlier steps in the immune response.

Macaulay noted that Viron's VT-111 acts on innate immune cells that are activated early during graft rejection and thus is likely to work upstream of any B cell mechanisms implicated in the two studies.

The compound, a myxoma virus-derived peptide that inhibits monocyte migration, is in preclinical development for renal transplant rejection. It has completed Phase IIa testing for acute coronary syndrome (ACS).

Macaulay also noted that the control groups in both studies were transplant patients on chronic immunosuppressant therapy.

"The first step is to understand what tolerance looks like."

—Laurence Turka, Beth Israel Deaconess Medical Center

“It is hardly surprising that some markers of immune cell presence and function are decreased” in these patients, he told *SciBX*.

Predictive power

The real value of the findings may be in identifying a larger group of allograft-tolerant patients for subsequent studies. Indeed, each team has devised a method of identifying tolerant patients using serological and gene expression data.

The European team found 10 genes whose expression is most significantly perturbed in tolerant patients compared with controls. Combined with measurements of certain immune cell ratios, the profile proved highly effective at identifying the tolerant patients within the European team's study cohort.

The American team narrowed their profile to just three genes and a cytological measurement of immature B cell levels.

To test the predictive power of their profiles, the two teams then swapped samples. Both methods were effective at identifying the patients known to be tolerant. Moreover, the European team's method suggested that 7 out of 54 control patients in the American cohort might in fact be tolerant.

According to Turka, the Immune Tolerance Network's next step will be to use the profiles in a larger, prospective trial.

“The next phase is a longitudinal and cross-sectional study of 100–200 patients post-transplant to see how many have the signature of tolerance,” he said. “If 10%–15% of the patients have these biomarkers, it would be wonderful.”

Turka said the eventual goal is to select patients for a prospective trial in which immunosuppressant therapy would be gradually withdrawn.

“These studies propose a rational way to discriminate patients that are going to be highly tolerant” of a graft, said Pangaea CEO José Jimeno. “These patients will be more amenable to reducing immunosuppressant dosage.”

Pangaea was founded in 2007 and is focused primarily on oncology biomarkers. The company has an exclusive option to license a patent on the American team's study that is co-owned by the University of California, the University of Pennsylvania and Emory University, which were the host institutions to some of the study's coauthors.

Hernandez-Fuentes said that Indices of Tolerance started a larger observational trial using its biomarker profile last September.

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—*Maria Hernandez-Fuentes,
King's College London*

She also noted that the gene expression profiles may be useful for testing the efficacy of tolerance-inducing drug candidates.

“Before these papers, there was nothing you could do to demonstrate that a drug makes you tolerant,” said Hernandez-Fuentes. “Now we have an endpoint” for such trials.

Uwe Janssen, head of business development at Miltenyi's diagnostics unit and a coauthor on the European study, said his company co-developed the microarray diagnostics procedure used in the study and thus has “early/preferred access” to a pending patent filed on the work by Indices of Tolerance.

Janssen said that Miltenyi continues to collaborate with Indices of Tolerance and is waiting for further validation of the method's predictive power in the study that began last September.

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