Color-coded tracking method helps scientists analyze outcomes of newly transplanted tissue

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A group of "color-coded" laboratory mice are providing researchers with a novel way of tracking T-cells, enabling them to visualize and monitor the cellular immune responses of transplanted tissue in real time. The new imaging system is described in the June issue of *Nature Medicine*, which appears on-line this week.

"These immune responses are a key consideration in developing strategies to improve transplant outcomes," explains co-senior author Terry Strom, MD, Co-director of the Transplant Institute at Beth Israel Deaconess Medical Center (BIDMC) and Professor of Medicine at Harvard Medical School (HMS). "The fate of a transplant following withdrawl of immunosuppressive therapy - either rejection or tolerance - is thought to be dependent upon the balance of destructive and protective T cells. With this new system, we can actually visualize this balance."

The acquisition of tolerance - a state in which transplanted tissue is not rejected by the body even in the absence of immunosuppressive therapy - is dependent upon several subsets of T cells, including protective regulatory T cells (Tregs) and destructive effector T cells (Teffs).

"The issue of whether newly transplanted tissue is attacked or protected is not a black-and-white situation," explains Strom. "Even when transplants are rejected, there will be some protective Treg cells present. And, conversely, in cases of tolerance - when the new transplant is

accepted - there will still be some aggressive Teff cells at the scene of the crime." But, because it has not been possible for scientists to readily distinguish these two T cell subsets in vivo, the relative importance of these different types of T cells in the induction and maintenance of transplant tolerance has been unclear.

To address this issue, the BIDMC transplant immunology team, led by Strom and Maria Koulmanda, PhD, Associate Professor of Surgery at HMS, first created two mouse models - one that would express natural Treg cells (nTreg) in a fluorescent green protein and another in which Teff cells express a fluorescent red protein. (A third color, yellow, also came into play when the red effector T cells commit to the induced Treg phenotype, expressing both red and green fluorescent proteins and thereby appearing as yellow cells. This approach enables ready distinction between natural and induced Treg cells.)

The BIDMC investigators then partnered with a group of physicists at Massachusetts General Hospital led by Charles Lin, PhD. Lin and his team had developed a novel imaging technique that coupled in vivo flow cytometry with endoscopic confocal microscopy.

"Genetically mismatched insulin-producing islet cells were transplanted beneath the thin capsule surrounding the animals' kidneys," explains Koulmanda. "Infiltration of T cells into the transplant was then visualized in untreated recipient mice in which vigorous rejection occurred. These results were then compared with mice that had received a short course of treatment enabling them to permanently accept the transplant [i.e. immune tolerance]."

Using the new imaging process, the authors were able to clearly see that the ratio of protective T cells to destructive T cells differed markedly in the transplant tolerant mice compared with the mice in which transplant tissue was rejected. Among the "rejection mice," red cells rushed into

the transplant far in advance of infiltration into the transplant by the tolerance hosts.

"As the events of rejection proceeded, the number of transplanted infiltrating T cells vastly exceeded those present in the tolerant transplants, even though the numbers of 'protective' yellow and green cells were equal in these groups at a later point in time," explains Koulmanda. "This is the first time that we have been able to monitor transplanted allograft tissue in a live lab animal. While static images of cells have been captured in the past, our new method captures much more than just random snapshots of the process."

"A picture really is worth a thousand words," adds Strom. "By enabling us to visualize this process, this new system has given us a clearer understanding of both quantitative and qualitative characteristics of the CD4 T cell response to allografts in rejecting and tolerized hosts. Fourteen days post-transplant, we were able to witness as costimulation blockade-based therapy inhibited infiltration by [the fluorescent red] Teff cells. Given the effectiveness of these tools, we hope to construct a road map such that we can create drug-free transplant tolerance for our patients in the future."

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