Cells predict onset of graft-versus-host disease in men receiving BMTs from female donors

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Stanford University School of Medicine investigators have identified a clutch of cells that—if seen in a male patient's blood after receiving a brand-new immune system in the form of a bone-marrow transplant from a female donor—herald the onset of chronic graft-versus-host disease, or cGVHD. In this devastating syndrome, the patient's tissues come under a vicious and enduring assault by the transplanted cells.

"The overwhelming majority of patients who have these cells in their blood either have or will develop cGVHD within one to three months," said David Miklos, MD, PhD, assistant professor of medicine and senior author of the new study, which will be published online Feb. 4 in Proceedings of the National Academy of Sciences. Until now there have been no good predictive indicators for the onset of cGVHD, he said.

The discovery of this easily measured marker in the blood could help guide new therapies designed to mitigate or prevent cGVHD, the primary adverse outcome of transplantation of bone marrow from one person to another.

Bone marrow transplants are most commonly used to treat leukemia and lymphoma, conditions incurred when a blood or immune cell, respectively, becomes cancerous and proliferates. Together, these diseases account for some 50,000 to 75,000 new cases annually in the United States.

Bone marrow transplantation involves first clearing a patient's body of his or her own immune cells and then transplanting bone marrow, the source of all blood- and immune-forming cells, from a tissue-matched donor. The new cells, which are free of cancer, repopulate the patient's bone marrow and eventually give rise to a functioning set of blood and immune cells, providing a lifelong cure.

But in about half of such transplant procedures, patients ultimately develop cGVHD, Miklos said. In the one-quarter of all these transplants that involve male recipients and female donors, the risk is even higher.

That's an intentional tradeoff: While female-to-male bone-marrow transplants put the recipient at 40 percent higher risk of either acute or chronic GVHD than sex-matched transplants, they also reduce the male recipient's risk of a cancer relapse by 35 percent.

Cancer cells are, at heart, unstable and make all kinds of bizarre proteins, fragments of which they tend to display on their surface—a red flag to the immune system. The new immune system is therefore especially vigilant for cancerous cells that somehow survived the effort to destroy them, putting the patient at risk of a relapse.

But the immune system attacks not only infectious bugs or cancerous cells but any cells that it perceives as "foreign," including healthy cells bearing surface features the immune system hasn't become accustomed to over the course of its long-term exposure to the body's various tissues. So, the occupying army of immune cells from the donor all too often mounts a vicious, enduring, all-fronts attack on the recipient's healthy tissues.

The standard treatment for cGVHD is to administer steroids, which can globally suppress the entire immune system. This therapy has its drawbacks: notably, a greatly increased vulnerability to infectious

disease, weight gain, osteoporosis, muscle weakness and severe mood swings. Plus, it doesn't always work or, often, becomes a lifelong requirement.

The early warning indicator Miklos' team found is a particularly configured kind of B lymphocyte, one of many cell types that compose our immune system and are routinely infused in a bone marrow transplant.

Until recently, B cells have not been commonly suspected to induce cGVHD, because the job they're most well-known for is producing antibodies, an array of secreted proteins similar to arrows with designer tips. These arrowheads' vastly varying shapes—by some estimates, as many as a quadrillion (the number one followed by 15 zeroes) in a single person's immune system—give antibodies a collective capacity to bind to virtually every other protein that may dot a foreign cell's surface. Antibodies can grab onto an infecting pathogen, for example, immobilizing it and flagging it for an all-out assault and likely destruction by a heavyweight hit squad of aggressive immune cells.

Even when not engaged in antibody production, every B cell has surface receptors whose shapes closely resemble the "designer tips" of the antibodies the cell or its progeny will ultimately produce and secrete, should it become active. It was this shared feature that permitted the first-ever association of a set of B cells with the onset of cGVHD, Miklos said.

Essentially all human cells package their genetic materials as 23 chromosome pairs, each composed of one maternally derived and one paternally derived member. In 22 of those pairs, both members are closely similar. One pair, however—the one that determines our sex—consists of two chromosomes that, in a woman, are closely similar (two copies of the X version) but, in a man, are as different as a pair of

unmatched socks (denoted X and Y).

Virtually every cell in a man's body contains a Y chromosome, and so all these cells display, on their surfaces, certain fragments of the proteins produced according to the instructions of that Y chromosome. Analyses of entire sequences of the human genome have predicted that nine of these proteins would differ from their X-chromosome-produced counterparts by 5 percent or more. Six of these so-called "H-Y proteins" have been found to trigger strong immune responses by a woman's immune system. One of them, known as DBY, and especially a small fragment of this protein called DBY-2, generates a particularly robust response.

In earlier studies, Miklos and his colleagues observed a telltale sign of B-cell involvement in cGVHD among men who had received cells from female donors: the presence, in these patients' blood, of antibodies directed at sections of H-Y proteins, especially DBY-2. But while these antibodies were highly associated with the development of cGVHD, they didn't precede its onset—they became noticeable soon after clinical symptoms first emerged—so they couldn't be the cause of the syndrome.

Then perhaps the B cells that would produce and secrete these antibodies were, even before they started doing that, involved in some other way in inducing cGVHD.

In the new study, the investigators found a way to conjugate a fluorescent dye to DBY-2-targeting receptors on B-cell surfaces, tagging those cells so they could be detected by a technique called fluorescence-activated cell sorting, or FACS. Leonard Herzenberg, PhD, professor emeritus of genetics at Stanford and a co-author of the new study, invented FACS in 1972 and has been instrumental in its continued development since then.

Miklos and his associates looked at blood from 28 male patients at Stanford Hospital who, starting in 2005, had received transplants of bone marrow from female donors and had agreed in advance to let researchers periodically draw their blood and store the samples in a freezer at the Stanford Bone Marrow Transplant Research Repository.

Years later, the researchers examined the blood samples retrospectively. When they looked at samples drawn roughly six months after the transplant, they observed that 16 of the 28 had detectable levels of DBY-2-targeting B cells, accounting for 0.5-1.0 percent of all the B cells. Considering the trillions of different possible receptor varieties that, in theory, might be present in all, that's a huge percentage.

Moreover, of those 16 patients, medical records showed that 15 had ultimately developed cGVHD. Six of them already had it by the time their blood was first drawn, so it's impossible to say whether the cells or the disease came first. The other nine didn't yet have cGVHD, but went on to develop it between a month and a year later.

Of those 12 whose blood did not show evidence of the presence of the DBY-2-targeting B-cells, only five ultimately developed cGVHD.

Miklos said he thinks this kind of assay might be useful in carefully monitoring patients for early warnings of impending cGVHD and, if the signs are there, beginning aggressive pre-emptive treatment. Miklos cautioned that larger, prospective studies are necessary to establish DBY-2's utility.

Miklos and his associates have shown in other studies that an injectable drug called rituximab (itself an antibody) that attacks B cells while sparing other immune-cell types can reduce the incidence of cGVHD in female-to-male-transplant recipients. Miklos has conducted four different clinical trials (including one ongoing National Cancer Institute-

funded study still enrolling patients) using rituximab along with steroids to treat cGVHD.

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