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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Bender Ignacio et al, page 503

Viromewide antibody responses after transplantation

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In this issue of *Blood*, Bender Ignacio et al provide preliminary evidence for using a recently developed multiplex unbiased array (VirScan) to decipher humoral response after transplantation and the potential for VirScan to improve donor selection.[1]

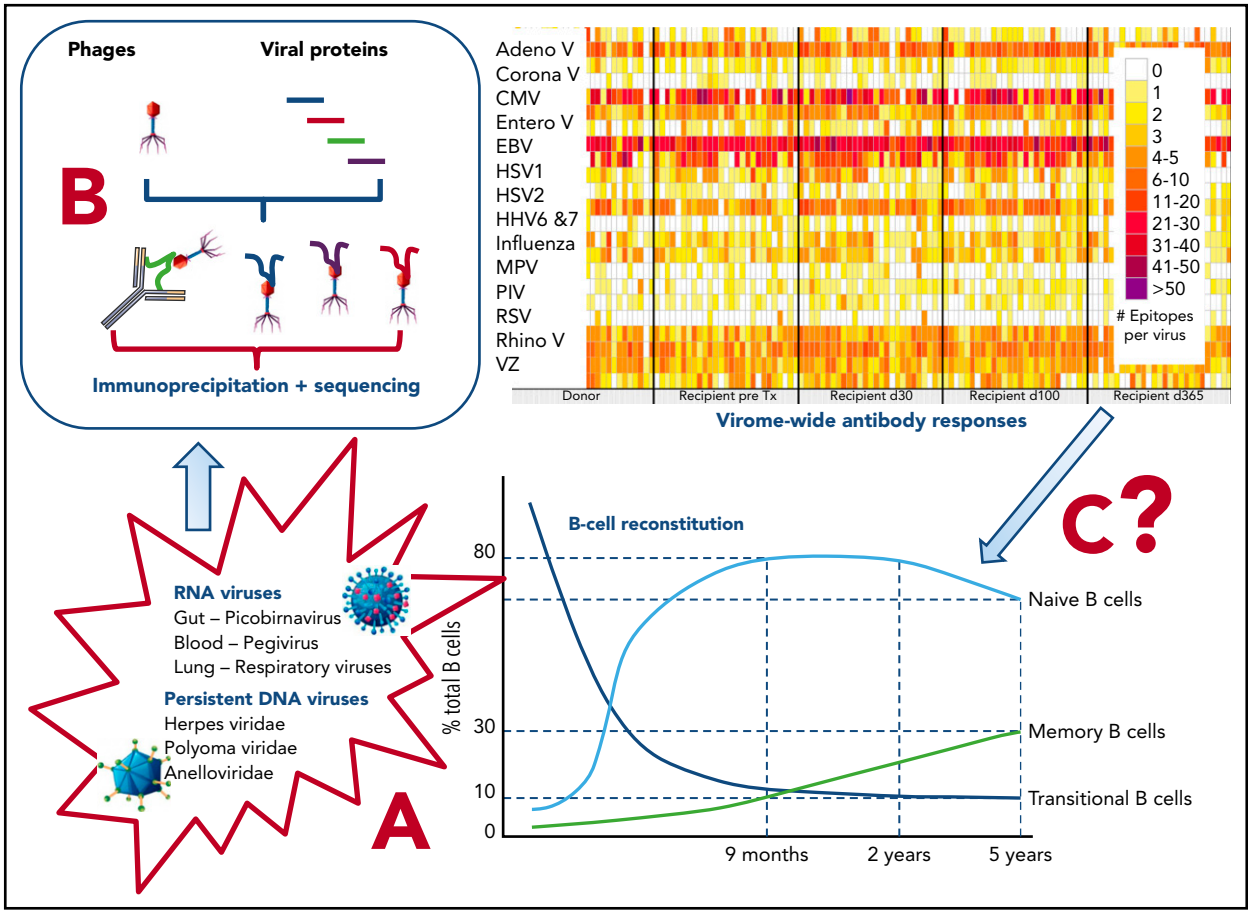
Transplant recipients are particularly susceptible to both viral reactivation and viral infection. After allogeneic hematopoietic stem cell transplantation (HSCT), immunosuppression used to prevent and treat graft-versus-host disease results in a heightened and prolonged risk for opportunistic viral infections[2] (see figure panel A). Bender Ignacio et al (from the Fred Hutchinson Cancer Research Center [FHCRC]; Seattle, WA) report the first analysis of HSCT recipients who were sequentially followed with VirScan. The seminal article describing VirScan was published in *Science* by Xu et al[3] in 2015. Basically, VirScan provides a comprehensive serologic profiling of human immunoglobulin G (IgG) to 206 viruses. This high-throughput technology allows detailed responses to viruses. It uses DNA microarray synthesis and bacteriophage display to create a representation of epitopes of the human virome. Immunoprecipitation and DNA sequencing are then used to characterize the peptides recognized as binding the IgG in the sample (see figure panel B). Since the original basic science article was published, the tool has slowly moved to translational research. Most recently, an article by Isnard et al[4] described the temporal virus serologic profiling of kidney graft recipients using VirScan. In that study, which involved 45 kidney transplant recipients, serologic profiling was performed on day 0 and at 1 year. Results were compared with an enzyme-linked immunosorbent assay and a polymerase chain reaction assay. Antibody responses to 39 of 206 species of virus present in the library were detected, and these responses were largely conserved during the year after transplant, regardless of immunosuppressive therapy.

Bender Ignacio et al studied 37 patient-donor pairs sequentially through myeloablative transplant, including samples from pretransplant and at days 30, 100, and 365 posttransplant (see figure panel B, right portion). Donor age, donor-recipient cytomegalovirus (CMV) serostatus, and use of corticoids influenced the diversity of the IgG antibodies repertoire at day 100. Somewhat counterintuitively, the IgG repertoire was similar to that of the donor at day 100 but similar to that of the recipient at day 365. As expected, gain or loss of epitopes to common viruses differed by donor and recipient pretransplantation serostatus, with highest gains in naïve donors to seropositive recipients, in particular for herpesviruses and adenoviruses. As previously reported,[5] CMV strongly shapes B-cell repertoire after allogeneic HSCT.

As always in good science, the Bender Ignacio article raises some questions. First, as a general comment, the authors used more sophisticated statistical analyses than those used in the kidney graft study.[4] The statistical methodologies used in their study were developed for analyses of the microbiome, and so-called "ecologic metrics" were developed to describe not only the total epitope score but also the diversity within each individual (eg, Simpson's D score that measures the α diversity), the donor-recipient antiviral response (β diversity), and the longitudinal estimate of distance between each donor-recipient pair using linear mixed-effects models, and to test the association between patient and transplant characteristics using generalized linear models. These refined biomathematical tools allowed the authors to perform a more nuanced analysis than that performed in the kidney transplant recipients (but much harder to read). As a cautionary note, these results are preliminary evidence because the number of patients studied is limited and the study involved only myeloablative conditioning. Moreover, the study is biased toward 1-year survivors and thus does not provide evidence about the IgG repertoire in patients who eventually succumbed as a result of viral-related diseases before 1 year.

What are the implications of those fascinating, although preliminary, results? The first implication is practical: VirScan may ultimately be a tool for screening and monitoring posttransplant virus infection. As stated above, it is too early to consider VirScan a routine method. In addition, as acknowledged by the authors, receiver operating characteristics of this synthetic virome to determine patient and donor serostatus is limited to viruses for which public epitopes and validated serologic methods are available (see supplemental Table 1 in the Bender Ignacio et al article for details). Furthermore, it should be remembered that VirScan analyzes only the IgG repertoire and does not investigate the Ig switch (Ig-M to Ig-G) that characterizes most recent viral infections.

The second perspective, in our opinion, is far more exciting. VirScan permits a greater



VirScan and immune reconstitution. (A) After transplantation, there is a bloom of virus reactivation, infection, and/or viral-related disease.[10] (B) Technical aspects of VirScan were originally published in Xu et al.[3] The right portion of panel B was adapted from the visual abstract of the article by Bender Ignacio et al. (C) This graph summarizes the reconstitution of the different B-cell subsets after transplantation. Adeno, adenovirus; Entero, enterovirus; EBV, Epstein-Barr virus; HHV6, human herpesvirus 6; MPV, metapneumovirus; HSV, herpes simplex virus; PIV, parainfluenza virus; Rhino, rhinovirus; RSV, respiratory syncytial virus; Tx, transplantation; V, virus; VZ, varicella zoster.

in-depth analysis of humoral immune reconstitution after transplantation. B-cell reconstitution studies after HSCT have come of age in the past 10 years (reviewed in Sarantopoulos and Ritz[6] and Socié[7]). Bender Ignacio et al address this point using only α and β metrics to correlate the IgG repertoire with 1-year total B-cell reconstitution (see supplemental Figure 4 in the article by Bender Ignacio et al for details). However, B-cell reconstitution is far more complex than could be ascertained from total B-cell counts (see figure panel C) (reviewed in Sarantopoulos and Ritz[6] and Socié[7]). The early B-cell reconstitution is dominated by transitional B cells that are pregerminal center, nonswitched B cells. The naïve B-cell population (that does not secrete Ig) emerges only from 9 months to 1 year after transplantation, and it takes months (up to 2 years) to fully reconstitute memory B cells (and as an assumption, plasma cells, for which few if any immune reconstitution data are available).

Finally, the authors assumed that the average half-life of IgG from the recipient was 26 days and thus surmise that any significant level of virus-specific IgG should come from the donor. This assumption can be challenged because the allotype of the IgG has not been studied (although it is weakly polymorphic) after HSCT. Previous work by the FHCRC on hemagglutinin showed that recipient IgG can persist much longer than 1 month,[8] and in 2007, a study demonstrated that humoral immunity to common viral and vaccine antigens can persist for decades (antibody half-life against rubella [114 years], Epstein-Barr virus [11.5 years], and varicella zoster virus [50 years]).[9]

In the near future, correlating in-depth cell phenotyping through mass cytometry and the B-cell receptor molecular rearrangements with results of the VirScan will be of major scientific interest.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Mascarenhas et al, page 525

Drug development challenges in polycythemia vera

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In this issue of *Blood*, Mascarenhas et al report on the safety and efficacy of oral idasanutlin in high-risk patients with polycythemia vera [PV]) with the goal of targeting hematopoietic stem cells and progenitor cells (HSC/HPCs) in this indolent malignancy.[1]

PV is a myeloproliferative neoplasm (MPN) characterized by expansion of the red blood cell mass. The majority of patients have a point mutation in the JAK2 gene in exon 14 or 12. Initial treatment of this disease aims to decrease the proliferation of blood cells and the risk of embolic and cardiovascular events. Therapies most often used first include phlebotomy or hydroxyurea. These therapies accomplish the goal of lowering blood counts but do not treat the underlying disease or protect against progression to myelofibrosis or the development of acute leukemia.

Interferon-α2a, an alternative therapy may not protect against progression either, but it can induce molecular remissions that are durable.[2] The clinical activity of interferon-α2a may be partially attributable to the upregulation of *TP53* activity.[3] JAK2V617F mutations increasing MDM2 protein translation by upregulating the La antigen which in turn alters p53 responses to DNA damage.[4] In the presence of elevated MDM2 protein levels, *TP53* messenger RNA levels are lower as compared with normal $CD34^+$ cells. MDM2 and MDM4 can each independently bind to p53 and block its transcriptional activity, and together have ubiquitin ligase activity.[5,6] In an earlier publication, Mascarenhas and collaborators showed that MDM2 is upregulated in PV 34^+ stem cell progenitors and that nutlins (a class of drugs that inhibit MDM2 activity) are capable of depleting mutated PV HSC/HPCs.[7] MDM inhibition results in upregulation of *TP53* which may allow for curative therapy.

$JAK2V617F^+$ patients were treated with idasanutlin at 2 dose levels on this phase 1 expansion trial to evaluate the safety and efficacy of this drug. Patients were treated for 5 days out of each cycle with the study drug. For patients who achieved a response to treatment, subsequent cycles were not given until prespecified hematologic parameters were met. Those patients who were tolerant of the drug but did not achieve a response with single-agent idasanutlin after 6 cycles were eligible to receive combination therapy with idasanutlin combined with interferon-α2a.

The overall response rate was 58% (7/12) for single-agent idasanutlin and 50% (2/4) for the combination arm with interferon-α2a. The median duration of response was 16.8 months. Scores on the MPN Symptom Assessment Form were improved with a maximum total symptom score (TSS) reduction of 81.5%. Idasanutlin therapy was associated with a 43% mean reduction in the JAK2V617F variant allele frequency in all patients except one, who had a p53 mutation. Toxicities of the drug were mainly nonhematologic. Five patients experienced grade 3 adverse events, mainly of the gastrointestinal tract during days 3-6 of drug administration. Eighty-three percent of patients experienced grade 1 or 2 nausea and needed a 3-drug antiemetic regimen consisting of ondansetron, decadron, and lorezepam to control symptoms during the 5 days of study drug administration. Four patients withdrew from the study, and 3 patients were taken off study by the investigator.

This study illustrates some important points about clinical trials in patients with PV. Given that PV is a rare disease with prevalence in 2003 of 22 per 100 000, the number of patients eligible for PV clinical trials is small.[8] Combined with the fact that only 5% of adults in the United States participate in clinical trials, there are few potential participants, and, given the small numbers enrolled, it is difficult to fully assess safety and efficacy of drugs. There are few resources to increase clinical trial participation in the United States or to encourage those with rare diseases to enroll in higher numbers. To make progress, there needs to be a funded national network to connect numerous study sites across the country and build consensus among sites to look at individual drugs for phase 1 to 2 testing.

The authors consider this agent promising as responses were seen in the majority of patients with only grade 1 to 2 nausea responding to treatment with a 3-drug antiemetic regimen during the 5 days of treatment. Although physicians often dismiss grade 1 to 2 toxicities as "tolerable" in a drug used to treat a neoplasm, they may not be acceptable to a patient population that may need to take the drug for many years. In this study, even though there were excellent hematologic responses and improvements in TSS on the MPN-SAF, the main reason patients discontinued the drug was because of the gastrointestinal toxicity. Even low-grade toxicity can contribute to decreased day-to-day quality of life, and the tradeoff for patients may not be worth a better long-term outcome. With long-term administration, even grade 1 to 2 toxicities might not be tolerable in PV patients.