CORRESPONDENCE

Genomic Mismatch at *LIMS1* Locus and Kidney Allograft Rejection

TO THE EDITOR: Steers et al. (May 16 issue)¹ describe the association between donor–recipient genomic collision at the *LIMS1* locus and the risk of kidney allograft rejection, which is mainly T-cell mediated. In addition, anti-*LIMS1* antibodies were detected in patients with allograft rejection who had homozygous deletions. In vitro, these antibodies are cytotoxic.

This mechanism is similar to how mismatching of human leukocyte antigen (HLA) can elicit a cellular immune response and the formation of de novo donor-specific HLA antibodies that can target mismatched HLA molecules on allograft endothelial cells.² In such cases, this process leads to microcirculation inflammation, the histologic hallmark of antibody-mediated rejection.

However, there is a potential pathogenic difference between anti-*LIMS1* and anti-HLA antibodies. In normoxic conditions, intrarenal *LIMS1* expression occurs mainly in tubular epithelial cells. However, without *LIMS1* expression on the surface of vascular endothelial cells, circulating anti-*LIMS1* antibodies could remain harmless.

Microcirculatory inflammation is often encountered without the presence of detectable donor-specific anti-HLA antibodies and may be explained by the action of non-HLA antibodies.^{3,4} Can the authors elaborate on whether patients with anti-*LIMS1* antibodies had such inflammation? Without such an association, screening for anti-*LIMS1* antibodies in patients with unexplained microcirculatory inflammation seems less relevant.

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No potential conflict of interest relevant to this letter was reported.

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TO THE EDITOR: The study by Steers et al. shows that the risk of kidney-transplant rejection is increased if a recipient carries a homozygous deletion in *LIMS1* but receives an allograft from a donor who has at least one normal allele. Although the authors state that hypoxia induces *LIMS1* expression, they do not adjust their analyses for ischemia time, nor do they assess the interaction between risk genotype and ischemia time. Organ procurement, transport, and implantation provoke allograft ischemia, which in turn induces the immunogenic *LIMS1* expression. Thus, allograft ischemia is likely to enhance alloreactivity in recipients with *LIMS1* genotype mismatching. Several investigators have described an increased risk of allograft rejection and HLA-antibody production with prolonged ischemia time,^{1,2} which is explained by enhanced alloreactivity during ischemia.³ If limiting the duration of ischemia would indeed modify the risk of allograft rejection in recipients with the risk genotype, it would be prudent to make every effort to limit ischemia time in such recipients.

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TO THE EDITOR: Steers et al. identified LIMS1 as a minor histocompatibility antigen associated with the development of de novo anti-LIMS1 antibodies and an increased risk of acute rejection in kidney recipients. Our recent study¹ of early acute rejection with features of antibody-mediated rejection in the absence of donor-specific anti-HLA antibodies complements the results of the study by Steers and colleagues. The *LIMS1* risk genotype appeared to be primarily associated with very early rejections of unknown histologic type. Using the same protein array, we detected anti-LIMS1 antibodies in 9 of 20 patients with antibody-mediated rejection and in none of 10 controls (P=0.01).

This finding suggests that anti-LIMS1 antibodies may be associated with histologic features of antibody-mediated rejection. In our patients, anti-LIMS1 positivity was observed in pretransplantation serum samples, which suggests that anti-LIMS1 antibodies may be preformed, even in patients without previous sensitization events (as in 3 of 9 patients). In 5 of the 9 patients with antibody-mediated rejection who had anti-LIMS1 antibodies, we also detected a strong seroreactivity against glomerular endothelial cells. This finding suggests that if the presence of anti-LIMS1 antibodies contributes to antibody-mediated rejection, other antiendothelial-cell antibodies² may also be involved, which illustrates a global dysregulation of humoral response.

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TO THE EDITOR: Steers et al. report no significant association between the *LIMS1* collision genotype and graft survival, despite a major association with acute rejection. We recently reported that genomewide mismatch in polymorphic residues in immune-accessible proteins was associated with graft survival.¹ Such an observation extends the current understanding of B-cell epitope mismatch from HLA (e.g., eplet score) to genomewide incompatibility. In our analysis, no single polymorphic variant mediated the risk of graft loss.

Additional evaluation of *LIMS1* in our cohort also showed a nonsignificant difference in the risk of graft failure in a comparison of donor-recipient pairs that had the collision genotype and those that did not have the genotype (adjusted hazard ratio, 1.33; 95% confidence interval, 0.63 to 2.83). Loss-of-function or copy-number variants, such as the *LIMS1* collision genotype, that result in the complete loss of gene expression may have a stronger effect on the alloimmune response than a single polymorphic residue.²

Whereas acute cellular rejection is driven by the recognition of non-self HLA by directly alloreactive T cells, antibody-mediated rejection is caused by donor-specific antibodies against HLA and non-HLA antigens.^{3,4} To better understand alloimmunity of *LIMS1*, it would be interesting to have more information regarding the timing of antibody occurrence in relation to the diagnosis of humoral and cellular rejection.

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1. Reindl-Schwaighofer R, Heinzl A, Kainz A, et al. Contribution of non-HLA incompatibility between donor and recipient to kidney allograft survival: genome-wide analysis in a prospective cohort. *Lancet* 2019;393:910-7.
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THE AUTHORS REPLY: We report that donor–recipient genomic collision genotypes at the *LIMS1* locus are associated with an increased risk of allograft rejection, predominantly of the T-cell–mediated subtype. The rejection events were associated with the presence of circulating anti-LIMS1 antibodies.

Callemeyn and Naesens raise important questions about the mechanisms and histopathological characteristics of allograft injury with anti-LIMS1 antibodies. As with any novel finding, more follow-up work is warranted. LIMS1 is expressed in human and murine endothelial cells, as shown in our study (Table S10 and Fig. S15 in the Supplementary Appendix, available with the full text of our article at NEJM.org), in the human protein atlas (www.proteinatlas.org),^{1,2} and in a recent article by Park et al.³ We currently do not have standardized data on microcirculatory inflammation. This issue should be investigated in future studies of anti-LIMS1–associated rejection, but alternative mechanisms of injury should also be considered, since there may be differences between rejection mediated by anti-LIMS1 antibodies and that mediated by anti-HLA antibodies.

Heylen and Thienpont point to the potential role of ischemia time and suggest exploring the interaction between the risk genotype and ischemia time in predicting rejection events. This is an important hypothesis that emerges from our results, and we agree that this issue will require further testing in the data sets with complete information regarding the duration of cold ischemia.

As described by Lamarthée et al., the detection of anti-LIMS1 antibodies in 45% of their patients who had early antibody-mediated rejection with no donor-specific anti-HLA antibodies represents an impressive finding. We are also intrigued by the presence of anti-LIMS1 antibodies in serum samples obtained before transplantation. Since LIMS1 is expressed in blood cells and other tissues,^{1,2} sensitization may occur in patients who are homozygous for the CNVR915.1 deletion after undergoing blood transfusion or childbirth. We therefore suggest genotyping of all donor–recipient pairs from this study and

checking their antecedent medical history. The detection of a strong seroreactivity against glomerular endothelial cells may be partially explained by anti-LIMS1 reactivity, since, as we mentioned above, the antigen is expressed by the glomerular endothelium. Nevertheless, the presence of additional antiendothelial antibodies and a more global dysregulation of humoral response is also possible.

Finally, we are pleased that Reindl-Schwaighofer et al. observed a similar trend for allograft survival in the *LIMS1* collision genotype, notwithstanding a much smaller sample size. We also congratulate Reindl-Schwaighofer and colleagues from the iGeneTRAiN (International Genetics and Translational Research in Transplantation Network) consortium for the recent report on the effects of donor–recipient mismatch in missense variants in transmembrane and secreted proteins with respect to kidney allograft survival.⁴ Similar to our study, this report contributes to the growing evidence regarding the role of genetic factors beyond HLA in determining allograft outcomes. We agree that some mismatched loci may have larger effects than others, and the injurious mechanisms may differ according to the combinations of mismatches in any given donor–recipient pair. Ultimately, the question of allograft survival will need to be addressed in adequately powered studies with long-term outcome data, which will require a larger multicenter effort.

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Since publication of their article, the authors report no further potential conflict of interest.

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