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To the editor:

Significantly higher frequencies of alloreactive CD4$^+$ T cells responding to nonpermissive than to permissive HLA-DPB1 T-cell epitope disparities

Increasing evidence suggests that donor-recipient disparities for human leukocyte antigen (HLA)–DPB1 can be of clinical importance in unrelated hematopoietic stem cell transplantation (HSCT).[1] Two overlapping algorithms for functional T-cell epitope (TCE) matching involving 3 (TCE3) or 4 (TCE4) groups of DPB1 alleles have previously been shown to be significantly predictive of survival after 10/10 and 9/10 matched unrelated HSCT.[2,3] In both TCE3 and TCE4, nonpermissive mismatches are directed against 2 groups of immunogenic antigens encoded by DPB1*09:01, 10:01, 17:01 (TCE3/4 group 1) and DPB1*03:01, 14:01, 45:01 (TCE3/4 group 2), respectively.[2,3] In TCE3, all other frequent DPB1 alleles including DPB1*02:01, 04:01, 04:02 and others are classified as poorly immunogenic TCE3 group 3, and DPB1 mismatches against these alleles are predicted to be permissive.[2] In TCE4, TCE3 group 3 is further subdivided into 2 separate groups comprising DPB1*02 (TCE4 group 3) and the other alleles (TCE4 group 4), with intermediate and poor immunogenicity, respectively.[3]

Rutten and colleagues have recently shown that T-cell responses could be obtained against DP antigens from all 4 groups,[4,5] thereby confirming the observations that led to the discovery of the DP locus by primed lymphocyte testing,[6] as well as those obtained later in mixed lymphocyte reactions (MLRs).[7,8] Interestingly, Rutten and colleagues observed high levels of cytokine production by CD4$^+$ T cells in response also to autologous DP molecules presumably presenting minor histocompatibility antigens,[5] suggesting that the HeLa cell transfectants expressing DP but not other class II antigens used in their experiments may not quantify physiologic frequencies of alloreactive T helper cells, which increase substantially with the number of mismatched HLA-DP alloantigens in classical in vitro assays.[9,10]

Here, we have quantified the frequency of alloreactive CD4$^+$ T cells responding to permissive or nonpermissive TCE3 or TCE4 DP mismatches, in classical MLRs between peripheral blood mononuclear cells (PBMCs) of responder (R)–stimulator (S) pairs matched for 10/10 of the non-DPB1 alleles. When S presented both a permissive and a nonpermissive mismatch, the percentage of responding CD4$^+$ T cells was more than 10-fold higher against the nonpermissive (DPB1*09:01, 10.65%) compared with the permissive mismatch (DPB1*04:02, 0.88%; Figure 1A), and this result was highly reproducible in 3 independent experiments (data not shown). Importantly, in 24 MLRs, we found a consistently higher percentage of CD4$^+$ T cells responding to nonpermissive DPB1 mismatches according to TCE3 (n = 9; mean 10.13% ± 7.51%; Figure 1B left panel) or TCE4 (n = 14; mean 7.72% ± 6.96%; Figure 1B right panel), compared with permissive mismatches according to TCE3 (n = 15; mean 2.34% ± 2.82%; Figure 1B left panel) or TCE4 (n = 10; mean 1.81% ± 2.82%; Figure 1B right panel). In the Kruskal-Wallis analysis of variance, this difference was significant both for TCE3 ($P < .05$) and TCE4 ($P < .05$). Responses against DPB1*02 (TCE4 group 3), classified as permissive for TCE3 but nonpermissive for TCE4, were significantly lower than those against TCE3/4 groups 1 and 2 (10.13% ± 7.51% and 3.39% ± 2.8%, respectively; $P = .04$, Mann-Whitney test) but higher than those against TCE4 group 4 (1.81% ± 2.82%), resulting in no significant net effect on the predictive value of TCE3 and TCE4.

Our data provide, for the first time, in vitro evidence for differential immunogenicity of DPB1 according to our algorithms. Interestingly, ex vivo evidence was previously reported by Rutten and colleagues[4] who showed that in 2 patients after 10/10 matched HSCT, the number of T cells responding to mismatched DP alloantigens was highest for TCE3/4 group 2 (2.72%), lower for TCE4 group 3 (1.08%), and lowest for TCE4 group 4 (0.41%). Further work is needed to determine the molecular and cellular basis of our algorithms, including the role of the DPα chain.

Federico Sizzano
Unit of Molecular and Functional Immunogenetics (UMFI), Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

Laura Zito
UMFI, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

Pietro Crivello
UMFI, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

Roberto Crocchiolo
Biostatistics Unit, Department of Health Sciences (DISSAL), University of Genova, Italy; and Bone Marrow Transplantation Unit, Department of Hematology, Institute Paoli Calmettes, Marseille, France

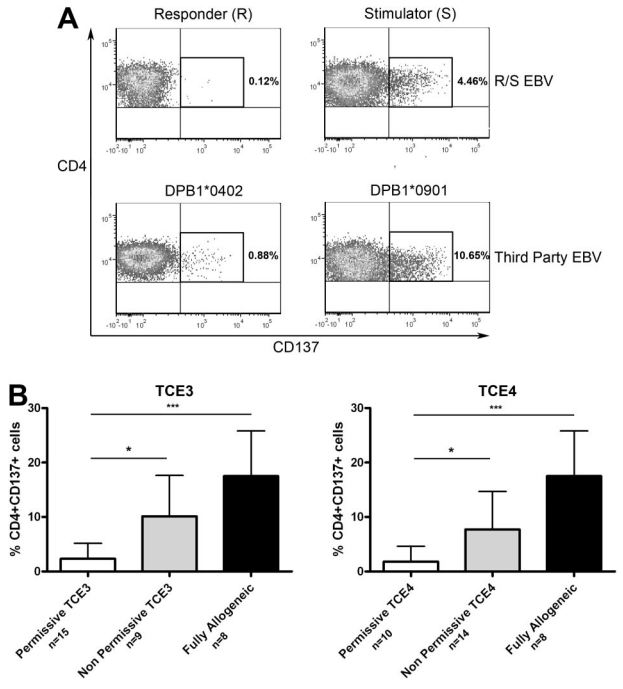

Figure 1. Quantification of alloreactive CD4$^+$ T cells responding to permissive or nonpermissive DPB1 TCE3 or TCE4 disparities. Classical 1-way MLRs were set up between R-S pairs of unrelated volunteers selected for the same patient and matched between each other for 10/10 of the HLA-A, -B, -C, -DRB, and -DQB1 alleles, but mismatched for -DPB1. R cells consisted of peripheral blood mononuclear cells (PBMCs), while S cells in most cases were PBMCs depleted of CD3$^+$ T cells, at a ratio of 1:1. After 2 rounds of stimulation in the presence of 150 IU/mL IL-2, CD4$^+$ T cells were rechallenged overnight with B lymphoblastoid cells (BLCLs) from R, S, or from third-party donors sharing only 1 mismatched DPB1 allele with S. Responding T cells were quantified by FACS analysis for surface expression of the activation marker CD137. In several cases, CD4$^+$ T cells expressing CD137 upon challenge with DPB1 typed third-party BLCLs were FACS-sorted and their specificity for the relevant DP alloantigen was confirmed (not shown). (A) Exemplative analysis of alloreactive CD4$^+$ T cells responding to a permissive or a nonpermissive DPB1 mismatch on the same S cell. R and S cells carried DPB1*02:02, 04:01 and DPB1*04:02, 09:01, respectively, and thus S cells presented 1 TCE3/4 permissive (DPB1*04:02) and 1 TCE3/4 nonpermissive (DPB1*09:01) mismatch. (B) Mean percentage of CD4$^+$ T cells responding to permissive or nonpermissive DPB1 mismatches according to TCE3 (left panel) or TCE4 (right panel), in a series of 24 MLRs. At the moment of testing, cultures contained a mean of 53.75% $\pm$ 24.94% CD4$^+$ T cells. The mean percentage of T cells expressing CD137 in response to autologous R-BLCLs was 2.06% $\pm$ 1.62%. The percentage of T cells responding specifically to allogeneic read-out BLCLs was calculated as the total percentage of CD137$^+$ T cells after allogeneic stimulation minus the percentage of CD137$^+$ T cells after autologous stimulation. Fully allogeneic R-S pairs (n = 8) were used as positive controls and yielded a mean of 17.53% $\pm$ 8.28% specifically responding CD4$^+$ T cells, with a mean of 44.75% $\pm$ 25.17% CD4$^+$ T cells. Pairwise comparison of the results obtained in the different groups was performed by the Kruskal-Wallis test followed by the Dunn multiple comparison posttest. (Left panel) In the TCE3 permissive group (n = 15), the mismatched DPB1 allele expressed by S was encoded by DPB1*02:01 (n = 4), 02:02 (n = 1), 04:01 (n = 5), 04:02 (n = 3), 11:01 (n = 1), 13:01 (n = 1). In the TCE3 nonpermissive group (n = 9), the mismatched DPB1 allele expressed by S was encoded by DPB1*03:01 (n = 3), 09:01 (n = 2), 10:01 (n = 3) or 17:01 (n = 1). The frequency of CD4$^+$ T cells specifically responding to TCE3 permissive mismatches was significantly lower compared with TCE3 nonpermissive mismatches (*$P < .05$) and compared with fully mismatched third-party alloantigens (***$P < .001$). (Right panel) In the TCE4 permissive group (n = 10), the mismatched DPB1 allele expressed by S was encoded by DPB1*04:01 (n = 5), 04:02 (n = 3), 11:01 (n = 1), 13:01 (n = 1). In the TCE4 nonpermissive group (n = 15), the mismatched DPB1 allele expressed by S was encoded by DPB1*02:01 (n = 4), 02:02 (n = 1), 03:01 (n = 3), 09:01 (n = 2), 10:01 (n = 3) or 17:01 (n = 1). The frequency of CD4$^+$ T cells specifically responding to TCE4 permissive mismatches was significantly lower compared with TCE4 nonpermissive mismatches (*$P < .05$) and compared with fully mismatched third-party alloantigens (***$P < .001$).

Luca Vago
Hematology and Bone Marrow Transplantation Unit, Department of Oncology, and Laboratory of Experimental Hematology, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

Elisabetta Zino
UMFI, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

Katharina Fleischhauer
UMFI, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, Milano, Italy

The authors declare that approval was obtained from the San Raffaele Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

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Correspondence: Katharina Fleischhauer, UMFI, Division of Regenerative Medicine and Stem Cell Therapy, San Raffaele Scientific Institute, via Olgettina 60, 20132 Milano, Italy; e-mail: fleischhauer.katharina@hsr.it.

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