immunosuppressive therapy (n = 7), donor unavailable (n = 8), early relapse (n = 2), or other (n = 2). Hematologic relapse was 16% in MRD^- patients, 6% in MRD^+DLI^+ patients, and 63% in MRD^+DLI^- patients ($P < .001$; Figure 1); the actuarial 3-year survival in these 3 groups was 78%, 80%, and 26%, respectively ($P = .001$; Figure 1). Mortality due to acute GVHD following DLI was 12%. In multivariate Cox analysis, the MRD group predicted relapse ($P < .001$) and survival ($P = .01$), together with disease phase and chronic GVHD. In MRD^+ patients, DLI protected against relapse ($P = .003$) and improved survival ($P = .01$).

In conclusion, we confirm that MRD detected after transplantation is a significant predictor of relapse. Treatment of MRD with DLIs appears to protect against leukemia relapse, although caution with DLI dosing needs to be used because of the potential risk of GVHD.

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The authors declare no competing financial interests.

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References

1. Kolb HJ, Schattenberg A, Goldman JM, et al, for the European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86:2041-2050.
2. Weisser M, Tischer J, Schnittger S, Schoch C, Ledderose G, Kolb HJ. A comparison of donor lymphocyte infusions or imatinib mesylate for patients with chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation. Haematologica. 2006;91:663-666.
3. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304:1529-1533.
4. Sullivan KM, Weiden PL, Storb R, et al. Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. Blood. 1989;73:1720-1728.
5. Bacigalupo A, van Lint MT, Occhini D, et al. Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. Blood. 1991;77:1423-1428.
6. Locatelli F, Zecca M, Rondelli R, et al. Graft versus host disease prophylaxis with low-dose cyclosporine-A reduces the risk of relapse in children with acute leukemia given HLA-identical sibling bone marrow transplantation: results of a randomized trial. Blood. 2000;95:1572-1579.
7. Uzunel M, Jaksch M, Mattsson J, Ringden O. Minimal residual disease detection after allogeneic stem cell transplantation is correlated to relapse in patients with acute lymphoblastic leukaemia. Br J Haematol. 2003;122:788-794.
8. Ogawa H, Tamaki H, Ikegame K, et al. The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. Blood. 2003;101:1698-1704.
9. Bader P, Kreyenberg H, Hoelle W, et al. Increasing mixed chimerism is an important prognostic factor for unfavourable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? J Clin Oncol. 2004;22:1696-1705.
10. Miglino M, Berisso G, Grasso R, et al. Allogeneic bone marrow transplantation (BMT) for adults with acute lymphoblastic leukemia (ALL): predictive role of minimal residual disease monitoring on relapse. Bone Marrow Transplant 2002;30:579-585.
11. van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukaemia for detection of minimal reCorrespondence: Andrea Bacigalupo, Divisione Ematologia 2, Ospedale San Martino, Largo Rosanna Benzi 10, 16132 Genova, Italy; e-mail : andrea.bacigalupo@hsanmartino.it.

To the editor:

Killer cell Ig-like receptors CD158a and CD158b display a coactivatory function, involving the c-Jun NH_2-terminal protein kinase signaling pathway, when expressed on malignant $CD4^+$ T cells from a patient with Sézary syndrome

Sézary syndrome (SS) is an aggressive leukemic and erythrodermic variant of cutaneous T-cell lymphomas characterized by the presence of a clonal T-lymphocyte population in the skin, lymph nodes, and peripheral blood.[1] We recently identified KIR3DL2/CD158k as the first cell-specific marker for the evaluation of the circulating tumoral burden and for the follow-up of patients with SS.[2-4] We next investigated the expression of additional killer cell Ig-like receptors (KIRs) on the peripheral blood mononuclear cells (PBMCs) of patients with SS and detected the simultaneous expression of CD158a and CD158b on all malignant cells from a unique patient (P1; Figure 1A, left panel).

To study the relevance of CD158a and CD158b expression by P1 malignant cells, a long-term cell line was generated. The identity of the in vitro–derived clone with the circulating tumoral clone was assessed by characterizing the CDR3-size Vβ distribution and the T-cell receptor (TCR) Vβ/Jβ junction (Figure 1B). Cell immunolabeling indicated that the derived T-cell line corresponded to the major TCR-$V\beta8^+CD158k^+$ circulating clone, and similarly expressed CD158a and CD158b (Figure 1A, right panel).

The influence of KIRs on the proliferation of a control $CD4^+CD158b^+$ T-cell line and P1 cells was evaluated. On control T cells, CD158b engagement led to a dramatic inhibition of their CD3-induced proliferation. In contrast, coligation of CD3 and CD158a or CD158b on the P1 cell line or on PBMCs resulted in an increased proliferation when compared with CD3 triggering alone (Figure 1C).

Further expression analysis demonstrated that both CD158a and CD158b were expressed under their inhibitory (KIR-L) and activating (KIR-S) isoforms in P1 cells, while only CD158b KIR-L was found on normal $CD4^+CD158b^+$ T cells (Figure 1D). It has been established that inhibitory KIRs exerted their activity, when phosphorylated, through an interaction with a protein tyrosine phosphatase.[5] We observed that CD158b became efficiently tyrosine-phosphorylated in activated normal $CD4^+CD158b^+$ T cells, and consequently interacted with SHP-1. In contrast, no tyrosine-phosphorylated CD158a or CD158b, nor coprecipitated SHP-1, were detected in activated patient cells (Figure 2D). Note that equal levels of each isoform were recovered regardless of the cell activation status (Figure 1D, top panel). Thus, no inhibitory

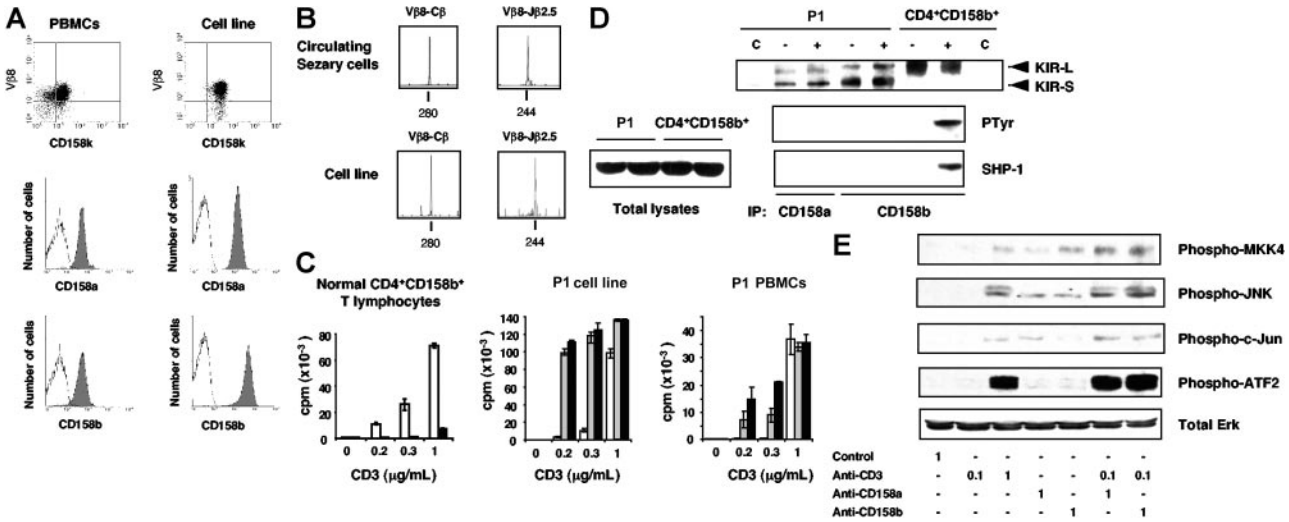

Figure 1. Expression and functional characterization of CD158a and CD158b on P1 malignant CD4+ T cells and an in vitro–derived T-cell clone. (A) PBMCs and the P1 cell line were subjected to a double immunostaining using a PE-conjugated anti–TCR-Vβ8 mAb, and an anti-CD158k mAb plus FITC-conjugated goat anti–mouse IgM antibodies (top panel). Alternatively, cells were stained with a PE-conjugated anti-CD158a (middle panel) or anti-CD158b (bottom panel) mAb. (B) The CDR3 size analysis of the TCR-Vβ8 transcript from P1 PBMCs (top panel) or derived cell line (bottom panel) was performed. Following total RNA extraction and reverse transcription, cDNA were amplified by PCR with Vβ8- and Cβ-specific primers. The unlabeled amplification products were elongated using a nested fluorescent Cβ and Jβ2.5 primers. The samples were subjected to electrophoresis and analyzed on an automated sequencer. (C) Expanded normal $CD4^+CD158b^+$ lymphocytes, the P1-derived cell line, or PBMCs were activated by incubation with an anti-CD3 mAb together with an isotype-matched anti-CD16 (white histogram), anti-CD158a (gray) or anti-CD158b (black) mAb. When necessary, the anti-CD16 mAb was used instead of the anti-CD3 mAb (concentration "0") alone, or in combination with an anti-KIR mAb. Concentrations of the anti-CD3 mAb used are indicated. Results are expressed as the mean of triplicates ± SD. (D) Sorted and expanded $CD4^+CD158b^+$ T cells from a healthy donor, or the P1-derived cell line, were surface biotinylated and left untreated (−) or incubated in the presence of vanadate (+). An aliquot of each NP40 cell lysate, corresponding to 5×10^5 cell equivalent, was collected, and immunoprecipitations were performed on the remaining samples using an anti-CD16 (C), anti-CD158a, or anti-CD158b mAb. The immunoprecipitates were separated by SDS–8% PAGE and transferred onto a nitrocellulose membrane. The immunoprecipitated receptors were first revealed by incubation of the blot with streptavidin-peroxidase, and an ECL detection system (top panel). The position of the short (KIR-S) and long (KIR-L) isoforms is indicated. After a dehybridization step, the membrane was reprobed with the antiphosphotyrosine mAb 4G10 (middle panel), stripped, and incubated with the purified anti–SHP-1 polyclonal antibodies (bottom panel). Total cell lysates from control or activated cells were similarly probed with the anti–SHP-1 antibodies to ensure protein expression. (E) Cells were incubated in the presence of control murine IgG, anti-CD3 and/or anti-CD158a or anti-CD158b mAb. Following activation, cell lysates were prepared and subjected to gel electrophoresis. Immunoblotting was then performed sequencially using the indicated antiphosphoprotein antibodies. Equal protein loading was verified by detection of total Erk1/2. The concentrations of the antibodies used for cell activation are given in micrograms per milliliter.

signaling was apparently generated following CD158a or CD158b triggering of P1 cells.

Stimulatory receptors usually interact with adaptor molecules to promote the downstream recruitment of Syk family protein tyrosine kinases.[5] However, expression of the regular adaptor proteins DAP10 and DAP12 was undetectable in P1 cells, and no ζ was found associated with CD158a or CD158b in activated Sézary cells (not shown). In $CD4^+$ T cells, CD158j/KIR2DS2 was identified as a costimulatory molecule using the DAP12-independent JNK pathway.[6] We observed that CD158a- or CD158b-mediated stimulation of P1 cells resulted in phosphorylation of MKK4 and JNK, and to a lesser extent, of c-Jun and ATF2 (Figure 1E). In addition, while a suboptimal CD3 activation of the cells did not lead to protein phosphorylation, the coengagement of CD158a or CD158b resulted in the detection, for all proteins tested, of phosphorylation levels equivalent to that reached upon optimal CD3-mediated activation (Figure 1E).

In conclusion, we showed that CD158a and CD158b could act as costimulatory receptors on Sézary cells through the recruitment of the DAP12-independent JNK pathway. The delivery of coactivation signals through both KIRs, specific for all HLA-C alleles, might therefore contribute to Sézary cell clonal outgrowth in vivo.

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Contribution: A.M.-C., D.H., N.O., N.R., and S.L.G. performed the experiments; A.M.-C., A.B., and M.B. designed the research and analyzed the data; A.M.-C. and A.B. wrote the paper.

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A.M.-C. and D.H. contributed equally to this work.

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References

1. Willemze R, Kerl H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. Blood. 1997;90:354-371.
2. Bagot M, Moretta A, Sivori S, et al. CD4(+) cutaneous T-cell lymphoma cells express the p140-killer cell immunoglobulin-like receptor. Blood. 2001;97:1388-1391.
3. Poszepczynska-Guigne E, Schiavon V, D'Incan M, et al. CD158k/KIR3DL2 is a new phenotypic marker of Sezary cells: relevance for the diagnosis and follow-up of Sezary syndrome. J Invest Dermatol. 2004;122:820-823.
4. Ortonne N, Huet D, Gaudez C, et al. Significance of circulating T-cell clones in Sezary syndrome. Blood. 2006;107:4030-4038.
5. Lanier LL. Natural killer cell receptor signaling. Curr Opin Immunol. 2003;15:308-314.
6. Snyder MR, Lucas M, Vivier E, Weyand CM, Goronzy JJ. Selective activation of the c-Jun NH2-terminal protein kinase signaling pathway by stimulatory KIR in the absence of KARAP/DAP12 in CD4+ T cells. J Exp Med. 2003;197:437-449.