Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: improvement of their immune activity by expression of CD33-specific chimeric receptors

Virna Marin,[1] Irene Pizzitola,[1] Valentina Agostoni,[1] Greta Maria Paola Giordano Attianese,[1] Helene Finney,[2] Alastair Lawson,[2] Martin Pule,[3] Raphael Rousseau,[4] Andrea Biondi,[1] and Ettore Biagi[1]

[1]Centro Ricerca "M. Tettamanti", Clinica Pediatrica Università Milano-Bicocca, Ospedale San Gerardo, Monza, Italy; [2]UCB Celltech, Slough, UK; [3]University College of London, London, UK; [4]Centre Leon Berard, Lyon, France

Citation: Marin V, Pizzitola I, Agostoni V, Attianese GMPG, Finney H, Lawson A, Pule M, Rousseau R, Biondi A, and Biagi E. Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: improvement of their immune activity by expression of CD33-specific chimeric receptors. Haematologica 2010;95(12):2144-2152. doi:10.3324/haematol.2010.026310

Supplementary Appendix

Design and Methods

Generation of cytokine-induced killer cells

CIK cells were prepared as previously described.[1] Briefly, peripheral blood mononuclear cells from healthy subjects were obtained after centrifugation of fresh blood on a density gradient using Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden). Cells were then resuspended in complete advanced RPMI medium (Invitrogen, San Giuliano Milanese, Italy). At the beginning of the culture, interferon (IFN)-γ (Dompè Biotec S.p.A, Milan, Italy) was added at 1000 U/ml. The next day, interleukin (IL)-2 (Chiron B.V, Emeryville, USA) and OKT-3 (Janssen-Cilag S.p.A., Cologno Monzese, Italy) were added at 300 U/mL and at 50 ng/mL, respectively, and cells were kept at the initial concentration of 3×10^6 cells/mL. Cells were then cultured for 21 days. Fresh medium and IL-2 were added weekly during culture and cell concentration was maintained around 0.5×10^6 cells/mL.

Chemotaxis and trans-Matrigel migration assays

Chemotactic migration assays were performed as previously described.[2] with 96-well Transwell insert (5-μm pore size; Corning Costar, Corning, Amsterdam, The Netherlands), adding 300 ng/mL of the chemokine CXCL12 (PeproTech, Rocky Hill, USA). Migrated cells in the bottom wells were enumerated after 1 h by quantitative flow-cytometry analysis. Results are expressed as the migration index of CIK cells in response to the chemokine versus the basal condition. Trans-Matrigel migration assay were performed as previously described.[3] Migrated cells in the bottom wells were enumerated after 3 h by quantitative flow-cytometric analysis.

Short-term cytotoxicity assay

The cytotoxicity of unmanipulated and anti-CD33.CAR-modified CIK cells against leukemic cells was evaluated as previously described[2] with a standard 4-h ^{51}chromium-release cytotoxicity assay. Radioactivity was detected by a β-scintillation counter (PerkinElmer Life Science, Boston, USA), as counts per minutes (cpm) and the percentage of specific lysis was calculated as previously described.[4] Experiments were performed in triplicate.

Long-term cytotoxicity assay

The killing activity of unmanipulated and anti-CD33.CAR-modified CIK cells toward leukemic cells was also determined after 6 day co-cultures in duplicate at 1:100 and at 1:200 effector:target (E:T) ratios, without exogenous IL-2, on a human bone-marrow derived stromal mesenchymal cell layer, as previously described.[5] After 6 days, cells were harvested, passed through a 19-gauge needle to disrupt residual mesenchymal-cell aggregates, stained with PE-anti-CD33 antibody and assayed by flow cytometry with a method specifically designed to enumerate leukemic cells recovered from culture, as previously described.[5]

Cell proliferation assay

CD33-specific proliferation was evaluated by 3H-thymidine (Amersham Pharmacia Biotech, Piscataway, USA) incorporation. Unmanipulated and anti-CD33.CAR-modified CIK cells (1×10^5) were co-cultured for 72 h with γ-irradiated HL-60 or primary AML cells, in triplicate at a 1:1 E:T ratio, without the addition of IL-2. Radioactivity (in cpm) was measured in a β-scintillation counter (Beckman Coulter, LS6500 multipurpose scintillation counter, Brea, USA). Data are expressed as a proliferation index), calculated as the ratio of cpm stimulated/cpm unstimulated conditions. As a control, the same assay was performed using the CD33-negative SUP-B15 cell line.

References

1. Schmidt-Wolf IG, Finke S, Trojaneck B, Denkena A, Lefterova P, Schwella N, et al. Phase I clinical study applying autologous immunological effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma. Br J Cancer. 1999;81(6):1009-16.
2. Marin V, Dander E, Biagi E, Introna M, Fazio G, Biondi A, et al. Characterization of in vitro migratory properties of anti-CD19 chimeric receptor-redirected CIK cells for their potential use in B-ALL immunotherapy. Exp Hematol. 2006;34(9):1219-29.
3. Zheng Y, Kong Y, Goetzl EJ. Lysophosphatidic acid receptor-selective effects on Jurkat T cell migration through a Matrigel model basement membrane. J Immunol. 2001;166(4):2317-22.
4. Montagna D, Maccario R, Locatelli F, Rosti V, Yang Y, Farness P, et al. Ex vivo priming for long-term maintenance of antileukemia human cytotoxic T cells suggests a general procedure for adoptive immunotherapy. Blood. 2001;98(12):3359-66.
5. Marin V, Kakuda H, Dander E, Imai C, Campana D, Biondi A, et al. Enhancement of the anti-leukemic activity of cytokine induced killer cells with an anti-CD19 chimeric receptor delivering a 4-1BB-zeta activating signal. Exp Hematol. 2007;35(9):1388-97.