To the editor:

Mystery solved: VSV-G-LVs do not allow efficient gene transfer into unstimulated T cells, B cells, and HSCs because they lack the LDL receptor

Vesicular stomatitis virus (VSV) G-protein pseudotyped lentiviral vectors (VSV-G-LVs) signify a major advancement in the gene and immunotherapy field as illustrated by successful clinical trials, for example, for Wiskott Aldrich Syndrome and leukodystrophies.[1]

Although VSV-G-LVs allow efficient transduction of nondividing cells,[2] they do not provide efficient transduction of quiescent T cells, B cells, and hematopoietic stem cells (HSCs), which hampers their application in gene and immune-therapy areas where conservation of cell phenotype is essential. Although these hurdles can be overcome in lymphocytes by LVs pseudotyped with measles virus envelope proteins (MV-LVs[3-5]), the reason as to why VSV-G-LVs were not efficient for gene transfer in these quiescent cells, and in particular in

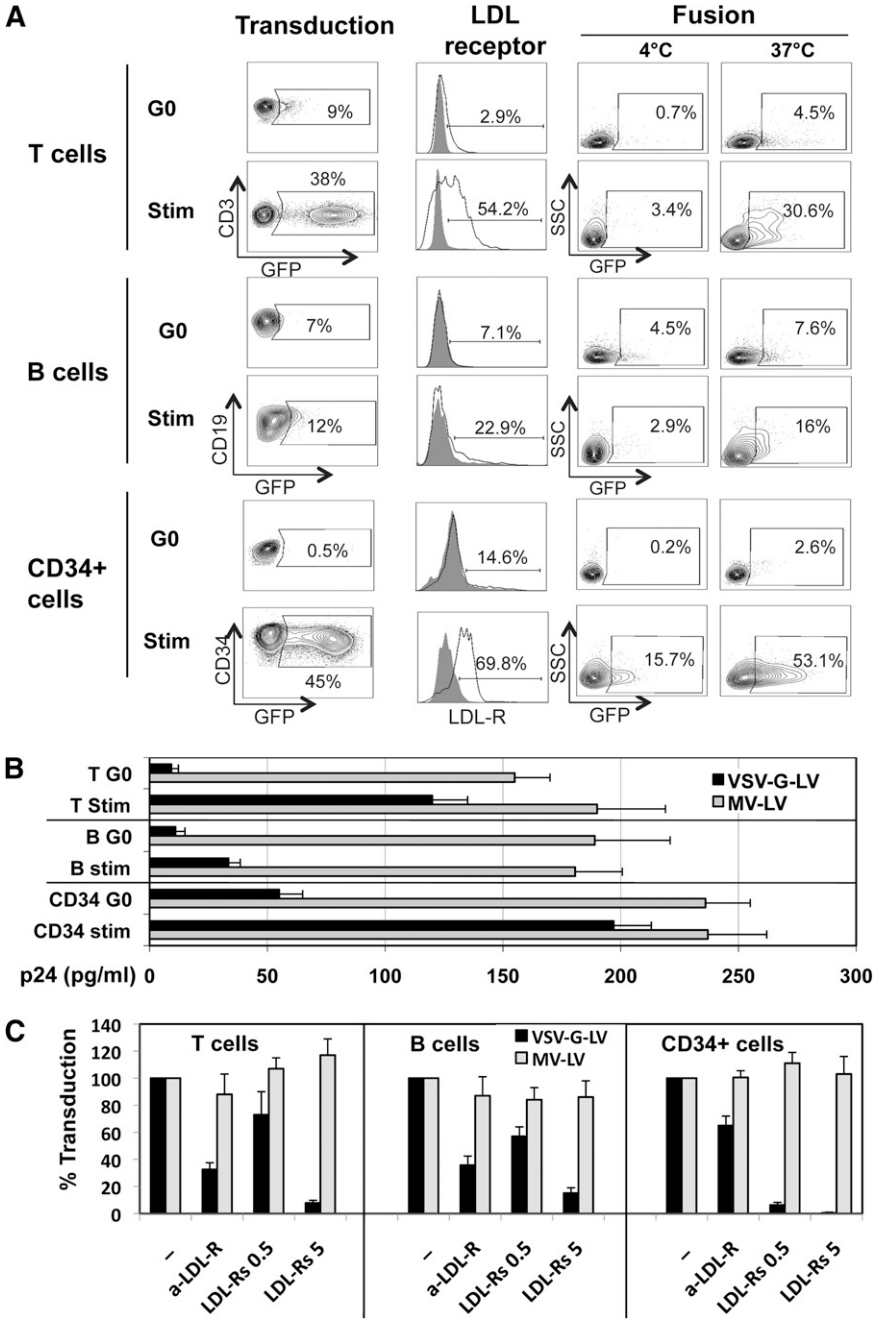

Figure 1. Low expression of LDL receptor on resting T cells, B cells, and CD34$^+$ cells limits VSV-G-LV binding, fusion, and transduction of these gene-therapy targets. (A) Unstimulated human T cells, B cells, and CD34$^+$ cells (G0) or 24-hour prestimulated (stim) T cells (anti-CD3 + anti-CD28 + IL-2), B cells (SAC + IL-2), and hCD34$^+$ cells (TPO + SCF + Flk-3L) were transduced with a GFP-encoding VSV-G-LV vector at an MOI = 50 (T and B cells) or MOI = 100 (CD34$^+$ cells) and GFP$^+$ cells were analyzed at day 3 posttransduction by FACS (see supplemental Methods, available on the *Blood* Web site); for LDL-R detection, freshly isolated or 24-hour prestimulated cells (see above) were incubated with the anti–LDL-R antibody (mouse mAb; R&D Systems) followed by staining with anti-mouse APC antibody (white open histograms), a control incubation with the latter antibody alone was performed (gray filled histogram); for fusion detection, freshly isolated or 24-hour prestimulated cells were incubated overnight with GFP gesicles[7] at 4°C to allow only binding or at 37°C to allow binding followed by fusion. The cells were then treated with trypsin to remove the GFP gesicles at the cell surface that did not fuse with the cells. (B) Equivalent quantities of VSV-G-LV or LV particles without envelope (measured by p24 content) were incubated with freshly isolated or 24-hour prestimulated cells (2^{E5} cells) for 1 hour at 4°C and then washed 4 times to remove unbound vector particles. The cells were pelleted and the cell-associated HIV capsid content (p24) was determined by ELISA (means ± SD; n = 3). The p24 signal for nonenveloped LVs was used as reference. (C) Entry through LDL-R was evaluated by blocking with a monoclonal antibody (C7, aLDL-R at 5 μg/mL; Santa Cruz Biotechnology) or by competition with soluble LDL receptor at 0.5 μg/mL (LDL-R 0.5; R&D Systems) or 5 μg/mL (LDL-R 5). A 1-hour preincubation of the prestimulated T cells, B cells, and CD34$^+$ cells with either blocking agent was performed before transduction with GFP-encoding VSV-G-LVs (MOI 50 for T and B cells; MOI 100 for CD34$^+$ cells) or MV-LVs (MOI of 10) for 48 hours, followed by FACS analysis for detection of GFP$^+$ cells (means ± SD; n = 3). aLDL-R, anti-low density lipid receptor antibody; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; IL, interleukin; mAb, monoclonal antibody; MOI, multiplicity of infection; SAC, staphylococcus aureus Cowan; SCF, stem cell factor; TPO, trombopoietin. Blood samples were obtained from healthy donors after informed consent and after local ethical committee approval in accordance with the Declaration of Helsinki.

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HSCs, remains unclear. Recently, Finkelstein et al revealed a long-kept secret of VSV by identifying its receptor, the low-density lipid receptor (LDL-R), explaining its broad tropism.[6] This finding prompted us to evaluate LDL-R levels on unstimulated T, B, and $CD34^+$ cells. Strikingly, we confirmed a very low expression of LDL-R, coinciding with VSV-G-LV–mediated poor transduction in these 3 cell lineages (Figure 1A). Stimulation of T cells through the T-cell receptor or of human $CD34^+$ ($hCD34^+$) cells with "early-acting cytokines" remarkably upregulated the LDL-R surface expression and permitted efficient VSV-G-LV transduction. In contrast, B-cell receptor stimulation augmented LDL-R expression only marginally, in agreement with poor VSV-G-LV transduction levels (Figure 1A and Frecha et al[4]). Binding of the different cell lineages with VSV-G-LVs was detected by incubation with the VSV-G-LVs followed by HIV capsid (p24) detection. VSV-G-LVs bound efficiently to stimulated T and $hCD34^+$ cells but not B cells and barely attached to their resting counterparts (Figure 1B). In contrast, MV-LVs efficiently attached to both stimulated and unstimulated cells (Figure 1B). Next, we used particles formed by VSV-G protein (gesicles[7]) incorporating high levels of green fluorescent protein (GFP) through a farnesylation tag to verify fusion of VSV-G protein with 3 cell lineages (Figure 1A, right panels). Resting T, B, and $CD34^+$ cells showed a poor GFP signal upon contact with GFP-loaded gesicles, while the GFP signal was evident for prestimulated cells, except for B cells (Figure 1A), confirming the presence of VSV and thus the VSV-G-LV receptor, LDL-R. Accordingly, VSV-G-LV transduction of resting T and B cells also resulted in very low levels of reverse-transcribed viral DNA.[4]

Finally, we confirmed the requirement for VSVG-LV entry and transduction through the LDL-R and its family members using an anti–LDL-R antibody or by competition with soluble LDL-R, resulting in reduction or almost complete inhibition of transduction, respectively (Figure 1C). In contrast, MV-LVs were not sensitive to these LDL-blocking or -competing agents. Interestingly, IL-7–stimulated T-cell VSV-G-LV transduction[8] coincided with LDL-R upregulation and was inhibited upon LDL-R blocking. Additionally, low-level transduction in resting cells was lost upon LDL-R blocking (data not shown).

In conclusion, although cellular postentry blocks may still play a role in VSV-G-LV transduction of resting T cells, B cells, and HSCs, we confirmed here that VSV-G-LV entry is compromised by the low expression of the VSV receptor LDL-R and its family members. Therefore, other LV pseudotypes (eg, MV-LVs) are more adapted for gene transfer in these invaluable resting gene-therapy targets.[9]

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To the editor:

Statin and aspirin use is associated with improved outcome of FCR therapy in relapsed/refractory chronic lymphocytic leukemia

Statins and aspirin are widely prescribed medications that have long been associated with improved survival outcome in patients with various types of cancers.[1,2] Both statins and aspirin were found to induce apoptosis of chronic lymphocytic leukemia (CLL) cells.[3,4] The intake of statins and aspirin was associated with reduced incidence of CLL.[5,6] However, statin intake did not affect treatment-free survival in patients with early CLL.[7,8] Whether statin or aspirin use will benefit patients with advanced CLL is unknown.

Therefore, we retrospectively investigated the clinical outcome of patients with relapsed/refractory CLL treated with salvage fludarabine, cyclophosphamide, and rituximab (FCR)[9] with or without concomitant statins, aspirin, or both. We analyzed 280 patients who received salvage FCR between 1999 and 2012. The patients' median age was 59 years (range: 31-84). The median progression-free survival (PFS) of all patients was 1.7 years, and the median overall survival (OS) was 4.0 years. Of the 280 patients, 58 patients received statins, aspirin, or both; 21 (8%) were taking aspirin only; 17 (6%) statins only; and 20 (7%) used both for at least 1 month prior to, during, and 1 month after salvage therapy. Among statin users, 15 patients (41%) were using atorvastatin, 12 patients (32%) were using simvastatin, 7 patients (19%) were using pravastatin, 2 patients (5%) were using rosuvastatin, and 1 patient (3%) was using lovastatin. Clinical characteristics of statin and/or aspirin users were similar to those of nonusers except for age. Patients on both statin and aspirin were 6 years older than nonusers ($P < .01$).

The overall response rate of patients receiving statins and aspirin concomitantly was superior (100%; 40% complete response, 60% partial response) to that of other patients (81% for aspirin-only users, 82% for statin-only users, and 72% for those who took neither drug; $P < .01$). Early death (during chemotherapy and up to 6 weeks afterward) was not observed in patients receiving aspirin, statins, or both but occurred in 6% of nonusers. Patients receiving both statins and aspirin had median PFS and OS of 6.1 and 9.2 years, respectively, compared with 1.6 years and 3.7 years in nonusers (PFS $P = .003$; OS $P = .05$; Figure 1). Compared with nonusers, patients who took both statins and aspirin had a 66% reduced risk of disease progression and a 60% reduced risk of death (PFS hazard ratio [HR] = 0.34, 95% confidence interval [CI] = 0.18-0.65, $P < .001$; OS HR = 0.40, 95% CI = 0.21-0.79, $P = .008$).

In a fitted multivariate model controlling for clinicopathological characteristics found to be statistically significant from univariate analyses including Rai stage, cytogenetic abnormalities, the number of previous treatments, refractoriness to fludarabine, IgV_H mutation status, β2-microglobulin, hemoglobin, platelet, lactate dehydrogenase, and creatinine level, use of both medications was also associated with a much more favorable outcome (PFS adjusted HR = 0.27, 95% CI = 0.14-0.53, $P \leq .001$; OS adjusted HR = 0.29, 95% CI = 0.15-0.58, $P < .001$), whereas single-agent use of aspirin or statins did not affect PFS or OS.

Our findings demonstrate for the first time that concurrent administration of statins and aspirin to CLL patients with relapsed/refractory disease receiving salvage FCR significantly improve both response rate and survival. This is consistent with previous preclinical studies suggesting the possible synergistic effect between statins and chemotherapy.[10] Therefore, a prospective study aimed at evaluating the effects of statins and aspirin in CLL patients receiving chemoimmunotherapy is warranted.

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