Down regulation of human natural killer cell–mediated cytolysis induced by blood transfusion: role of transforming growth factor-β_1, soluble Fas ligand, and soluble Class I human leukocyte antigen

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BACKGROUND: Human natural killer (NK) cells are thought to play a role in antiviral response and tumor immune surveillance. The molecular mechanisms of down regulation of NK-cell activity observed after red blood cell (RBC) transfusion is still undefined.

STUDY DESIGN AND METHODS: Both effects of blood transfusion (ex vivo) and supernatants (SNs) derived from RBC units unstored (RBC-0) or stored for 5 or 30 days (RBC-5 or -30, respectively) in vitro were analyzed on NK cell–mediated cytolytic activity.

RESULTS: We have found that NK cells isolated from transfused patients on Day 3 lysed the NK-sensitive target cells K562 to a lesser extent than before transfusion. This down regulation of NK-cell activation was evident also for NK-cell killing mediated through the engagement of NK cell–activating receptors as NKG2D, NKp30, NKp46, and CD16. Transfused patients reacquired NK cell–mediated cytolytic activity from Day 5 to Day 7 after transfusion. SN from RBC-30, but not from RBC-0 or RBC-5, strongly inhibited the generation of lymphokine-activated killer (LAK) cells and lysis of the NK-resistant target cell Jurkat in a dose-dependent manner. Transforming growth factor-β1 (TGF-β1) blocking antibodies partially restored the generation of LAK activity. In addition, the depletion of both soluble Class I human leukocyte antigens (sHLA-I) and soluble Fas ligand (sFasL) from SN of RBC-30 completely restored the generation of LAK activity.

CONCLUSIONS: Altogether, these findings would support the idea that blood transfusion–mediated down regulation of NK-cell activity is mediated by sHLA-I, sFasL, and TGF-β1.

It has become apparent that blood transfusion has generalized effects on a patient's immune system. The existence of deleterious clinical transfusion-related immunomodulation effects has not yet been confirmed;[1] but, on the other hand, these effects have been postulated to underlie the clinical observations that transfused patients have an increased postoperative bacterial infections as well as more frequent recurrences of malignant tumors.[1-5] Although the mechanisms of transfusion-related immunomodulation have been debated extensively, the exact mechanism(s) of this phenomenon has yet to be elucidated. A number of putative

ABBREVIATIONS: E : T ratio = effector-to-target ratio; GAM = goat antimouse; LAK = lymphokine-activated killer; NK = natural killer; RBC-0 = unstored red blood cell units; RBC-5 = red blood cell units stored for 5 days; RBC-30 = red blood cell units stored for 30 days; sFasL = soluble Fas ligand; sHLA-I = soluble Class I human leukocyte antigens; SN(s) = supernatant(s).

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This work was partially supported by grants from Compagnia del San Paolo n.2007.2065 to AP and AIRC2009 to MRZ (IG8727) and AP (IG8761).

Received for publication July 23, 2010; revision received November 8, 2010, and accepted November 9, 2010.

doi: 10.1111/j.1537-2995.2010.03000.x

TRANSFUSION 2011;51:1567-1573.

mechanisms have been hypothesized; among those, it has been shown that transfused red blood cell (RBC) units contain soluble mediators, released from white blood cells during storage, capable of down regulating the immune system of the recipient by acting on T-helper cytokine profile, natural killer (NK), and cytotoxic T-cell function.[1-5]

Among anti-tumor lymphocytes, NK cells would represent the most powerful cytolytic effector cells that we are provided with.[6] Indeed, NK cells are the peripheral lymphocyte subset able to lyse tumor cells like the K562 cell line without any prior sensitization and they acquire, when exposed to interleukin (IL)-2, a highly efficient anti-tumor cytolytic activity, named lymphokine-activated killer (LAK) activity, against most tumor cells.[6-9] Furthermore, the recent discovery of several activating receptors at the NK-cell surface as NKG2D, NKp30, and NKp46, besides CD16, have clearly indicated that NK cells in humans are specifically provided by a unique series of triggering surface receptors responsible for the elimination of different tumor target cells.[10]

We have previously reported that NK cells can produce transforming growth factor-β1 (TGF-β1) upon stimulation with soluble HLA-I after interaction with CD8 receptor expressed on NK cells.[11] This TGF-β1, in turn, can down regulate NK cell–mediated cytolytic activity and cytokine production. TGF-β1 is a multifunctional cytokine able to down regulate several immune-mediated functions and it is considered a relevant cytokine in tumor escape from immune system–mediated control.[11] Furthermore, we have shown that NK cells can be triggered to die by apoptosis through the interaction of soluble Class I human leukocyte antigens (sHLA-I) with corresponding CD8 receptor and this effect is mediated by FasL/Fas interaction at the NK cell surface.[12]

Herein, we show that transfused patients display an impaired NK cell–mediated cytolysis which can be reacquired on Days 5 to 7 after transfusion. This effect is also evident using the supernatant (SN) of RBC units stored for 30 days (RBC-30) in vitro. Further, we provide some evidence that TGF-β1, sHLA-I, and FasL are conceivably involved in mediating this immunomodulatory effect.

MATERIALS AND METHODS

Unstored RBC units, RBC units stored for 5 or 30 days, and SN(s)

Blood units were obtained from healthy volunteers and submitted to the standard procedure for the preparation and storage of RBCs according to the Council of Europe "Guide to the Preparation, Use and Quality Assurance of Blood Components" (15th ed., 2009). Briefly, whole blood (450 mL) was collected in plastic bags (triplicate bag system, Baxter, Le Chartre, France) with 63 mL of citrate-phosphate-dextrose in the primary bag. After centrifugation (10 min, 2720 × *g*, 20°C), RBCs were separated from plasma and transferred into the satellite bags, containing 80 mL of saline-adenine-glucose-mannitol. Aimed exclusively at the in vitro autologous experiments, two healthy subjects donated both RBCs and peripheral blood mononuclear cells (PBMNCs). Storage time for RBCs before transfusion and in vitro tests was 30 days. As control, in vitro evaluations were also performed immediately after blood donations (RBC-0) and after 5 days of storage (RBC-5). The SNs of RBCs to be utilized in functional assays were extensively dialyzed to remove the additive solutions and, in some experiments, were immunodepleted with anti-HLA-I–, anti-TGF-β1–, or anti-FasL–specific monoclonal antibodies (MoAbs) by immune-affinity column as described.[13]

Patients

Ten patients transfused with 1 unit of RBC-30 were analyzed for NK cell–mediated cytolysis. Three of these patients had been transfused a few days after hysterectomy (fibromatosis), and the other seven had been transfused during orthopedic interventions (knee protesis). Blood samples were analyzed before (basal, Day 0) and after transfusion (Days 3, 5, and 7) for NK cell–mediated activity. PBMNCs were isolated as described and cytolytic activity against the NK-sensitive target cell K562 was assessed as described.[14]

MoAbs and reagents

The anti-CD16 (NK1, IgG1) MoAb (10) and the anti-CD56 (TA181H12, IgG2a) MoAb were obtained as described.[15-17] The affinity-purified anti-CD3 MoAb (Leu4, IgG1), the anti-CD4 MoAb (Leu3a, IgG1), the anti-CD8 MoAb (Leu2a, IgG1), the anti-CD56 MoAb (Leu19, IgG1), and the unrelated MoAb (Clone MOPC-31C, IgG1) matched for isotype as control MoAb were from Becton Dickinson (Palo Alto, CA); the anti-FasL (NOK-1, IgG1) was from PharMingen International (San Diego, CA). The anti-NKp30 MoAb (Clone Z25, IgG1), the anti-NKp46 MoAb (clone BAB281, IgG1), in either purified azide free form or PE-conjugated, and the anti-human Fas MoAb UB2 were from Immunotech (Marseille, France); the anti-FasL MoAb Alf-2.1a was from Ancell Corp. (Bayport, MN), and the anti-Fas MoAb (M38, IgG1) was from American Tissue Culture Collection (Manassas, VA). Affinity-purified blocking anti-human TGF-β1 MoAb (Clone 9016, IgG1) and the anti-NKG2D (Clone MAB139, IgG1) were purchased from R&D System, Inc. (Minneapolis, MN). The affinity-purified goat anti-mouse (GAM) anti-isotype specific antiserum was from Southern Biotechnology (Birmingham, AL). Purified GAM anti-Ig(H+L) was purchased from ICN Biomedicals, Inc. (Aurora, OH); immunomagnetic beads were from Oxoid (Dynal A.S., Oslo, Norway); and recombinant IL-2 was from Chiron (Proleukin, Chiron Italia, Siena, Italy). Cells

were cultured in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (FCS, Sigma, Milan, Italy) and with glutamine and penicillin-streptomycin (Biochrom).

Indirect immunofluorescence

Single fluorescence staining was performed as described elsewhere.[17] Briefly, aliquots of 10^5 cells were stained with the corresponding MoAb or with an unrelated MoAb (Becton Dickinson) followed by PE-conjugated anti-isotype–specific GAM serum (Southern Biotechnology). Samples were analyzed on a flow cytometer (CyanADP, Beckman-Coulter, Milan, Italy) equipped with an argon-ion laser exciting PE at 488 nm. Data were analyzed using a computer program (Summit 4.3.01, Beckman-Coulter). Results are expressed as Log red mean fluorescence intensity (MFI) in arbitrary units (*x*-axis) versus number of cells (*y*-axis).

Isolation of NK cells and generation of LAK cells

NK cells were isolated with an NK isolation kit (RosetteSep, StemCell Biotechnologies, Vancouver, British Columbia, Canada) from heparinized blood of transfused patients indicated or healthy volunteers according to the kit protocol with some modifications. PBMNCs were isolated as previously described and RBCs were recovered and washed twice; highly purified NK cells were obtained from healthy volunteers, after depletion of monocytes by plastic adherence and addition of washed autologous RBCs to PBMNCs at 30:1 ratio, with an NK cell enrichment kit (RosetteSep, StemCell Technologies) and were greater than 98% pure as assessed by CD56 staining.[18] The resulting cell population was 70% to 98% CD16+ (range of eight different experiments) but 99% CD3–. Highly purified CD3– cells were cultured in 96-well U-bottomed microplates (Greiner Labortecknic, Nurtingen, Germany) with RPMI 1640 supplemented with 10% FCS in the presence of 10 ng/mL of IL-2 in a final volume of 200 µL/well for 5 days to generate LAK activity as described.[18]

Cytolytic assays

Cytolytic activity of CD3– CD16+ NK-cell populations was analyzed in a 4-hour ^{51}Cr-release assay against NK-sensitive tumor target cell K562 and the LAK-sensitive tumor target cell Jurkat.[19] Target cells were labeled with ^{51}Cr and used at an effector-to-target (E : T) ratio of 20:1 to 5:1, in a final volume of 200 µL of culture medium in V-bottomed microwells in a 4-hour ^{51}Cr-release assay. One-hundred microliters of SN was counted in a gamma counter and percentage of ^{51}Cr-specific release was calculated as described previously.[20] In the redirected killing assay the FcγR+ murine mastocytoma cell line P815 was used as target in the presence of saturating amounts (3 µg/mL) of the indicated MoAb or in medium alone at the E : T ratio of 1:1. In each experiment, an unrelated MoAb matched for the isotype (Becton Dickinson) and the anti-CD56 MoAb (Leu19) were used as MoAb, which do not trigger cytolysis as they do not recognize activating receptors.[11,20,21]

Determination of sHLA-I, soluble Fas ligand, and TGF-β_1 concentrations in RBC SN(s)

The concentrations of sHLA-I and soluble Fas ligand (sFasL) molecules were determined by double-determinant immunoassay in RBC SN or RBC immunodepleted, as previously described.[22,23] The concentrations of TGF-β1 were determined by double-determinant immunoassays utilizing a commercially available kit (Quantikine R&D System, Inc., Minneapolis, MN).

Statistical analysis

Data are expressed as mean ± SD. One-way analysis of variance with Bonferroni's posttest was performed using computer software (GraphPad Prism, Version 4.00 for Windows, GraphPad Software, San Diego, CA). Differences were accepted as significant when p values were less than 0.01.

RESULTS

Influence of blood transfusion on NK cell–mediated lysis

First, we analyzed whether NK cell–mediated tumor cell lysis was affected by blood transfusion. To this aim, we evaluated NK-cell activity before and after transfusion of a single RBC-30 unit. We found that the NK cell–mediated cytolysis of the NK-sensitive target cell K562 was strongly down regulated after blood transfusion compared to cytolysis before this procedure (Fig. 1A). The inhibitory effect was maximal on Day 3 (range of inhibition, 50%-80%; n = 6) and NK-cell activity was completely recovered on Day 7 after blood transfusion (Fig. 1A). Although not shown, the differences found in NK-cell activity were not dependent on a different number of CD3– CD56+ NK cells present in peripheral blood. Indeed, we found by flow cytometry analysis that in each patient the percentages of NK cells before and after blood transfusion were superimposable (not shown). The impairment of NK-cell activation was further confirmed by the reduction of NK-cell lysis of the FcγR+ target cell P815 in a redirected killing assay (Fig. 1B). Indeed, triggering of P815 lysis mediated by the engagement of several activating NK-cell receptors including CD16, NKG2D, NKp30, and NKp46 was inhibited after RBC-30 transfusion (range of inhibition on different triggering signals, 40%-60%). An unrelated MoAb (Fig. 1B) or an anti-CD56 MoAb (not shown) matched for the isotype did not induce triggering of cytolysis. On Day 7 after transfusion, NK cells were able to kill P815 target cells

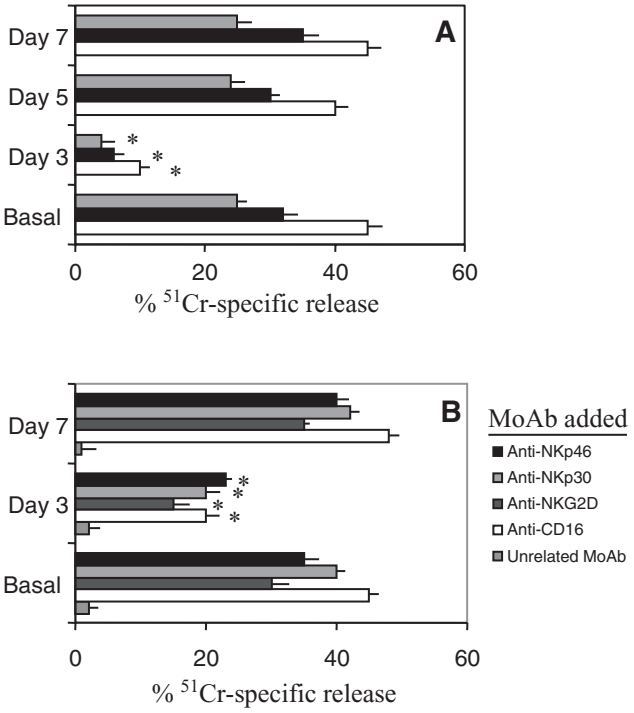

Fig. 1. Patients transfused display a reduced NK cell–mediated cytolytic activity. Peripheral blood NK cells from six patients transfused were analyzed for their ability of killing the NK-sensitive target cells K562 (A) or the murine mastocytoma P815 expressing the FcγR in a redirected killing assay (B). (A) NK-cell activity was analyzed at Time 0 (before transfusion, indicated as basal) or after 3, 5, or 7 days. Cytolytic activity was analyzed at the indicated E : T ratio. (B) At the same time points the triggering with different NK cell–activating receptors (NKp30, NKp46, NKG2D, and CD16) was also analyzed. Cytolysis with an unrelated MoAb of the same isotype of the antibodies to activating receptors is shown for comparison. The redirected killing assay was performed by adding the indicated MoAbs at the onset of the assay; an increment of lysis of P815 target cells indicates the activation of cytolysis in NK cells. Results are expressed as percentage of ^{51}Cr-specific lysis and are the mean ± SD of six independent experiments. *p < 0.001 versus basal. E : T ratios: (▩) 5:1; (□) 10:1; (■) 20:1.

similarly to that observed before transfusion (Fig. 1B). Although not shown, the inhibition of NK cell–mediated cytolysis observed was not related to ex vivo down regulation of these NK cell–activating receptors as demonstrated by flow cytometry analysis.

The generation of LAK cells is inhibited by SN from RBC-30

We also determined whether the generation of LAK cells in vitro could be affected by RBC-30 SN. To this aim, NK cells from healthy donors were stimulated for 5 days with 10 ng/mL IL-2 either in the presence or not of serial dilution (1:100, 1:1000, 1:10,000) of these SNs. Thus, LAK activity was analyzed using the LAK-sensitive leukemia target cell Jurkat. As shown in Fig. 2A, we found that the generation of LAK cells was strongly inhibited (50%-70% inhibition, range of six independent experiments) in the presence of SN from RBC-30. This effect was more evident

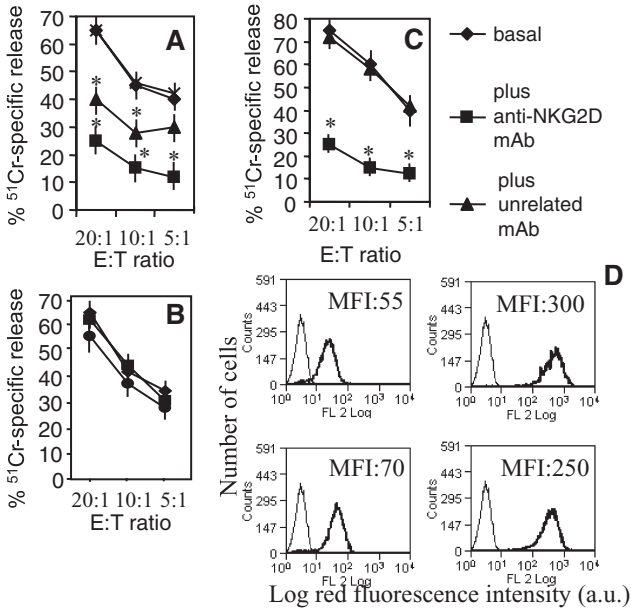

Fig. 2. RBC-30 SNs are able to inhibit the generation of LAK cell activity. (A) Cytolysis of Jurkat cells exerted by NK cells of healthy donors stimulated for 5 days with IL-2 in the absence or the presence of RBC-30 SN at different dilutions (◆, basal; ■, 1:100; ▲, 1:1000; ×, 1:10,000). (B) Cytolysis of Jurkat cells exerted by NK cells cultured as in (A) in the absence (◆, basal) or the presence of SN from RBC-0 (■) or RBC-5 (●) at 1:100 diluted. (C) Lysis of Jurkat cells is partly dependent on the engagement of NKG2D surface receptor (◆, basal; ■, plus anti-NKG2D MoAb; ▲, plus unrelated MoAb). This is demonstrated by the addition of anti-NKG2D MoAb (5 μg/mL) at the onset of the cytolytic assay and consequent blocking of Jurkat NK-cell lysis. The effect of the addition of an unrelated MoAb matched for the isotype is shown for comparison. Results are expressed as percentage of ^{51}Cr-specific lysis and are the mean ± SD of six independent experiments. *p < 0.001 versus basal. (D) Expression of NKG2D molecule on NK cells before (top left histogram) or after (bottom right histogram) stimulation with IL-2 or after stimulation with IL-2 in the presence of 1:100 RBC-30 SN (bottom left histogram) or with blocking anti-TGF-β1 MoAb, 1:100 RBC-30 SN, and IL-2 (bottom right histogram). In each subpanel is shown the histogram of negative control (thin line, stained with an unrelated MoAb matched for isotype) and the histogram of NKG2D (bold line). The results are expressed as red MFI in arbitrary units (a.u.) versus number of cells. In each subpanel is shown the MFI of NKG2D expression. Results are representative of six independent experiments.

at 1:100 while it disappeared when SN was diluted at 1:10,000. Since two healthy subjects donated either RBCs, stored 30 days before experiments, or PBMNCs, the same evaluations were performed in autologous modality, finding that both autologous and allogeneic RBC-30 SNs were effective at comparable dilution (not shown). Also SNs from RBC-0 or RBC-5 were assessed to determine whether they can affect LAK-cell generation. As shown in Fig. 1B, neither RBC-0 nor RBC-5 inhibited LAK-cell generation. As lysis of Jurkat cells is partly dependent on the NKG2D-mediated engagement (Fig. 2C), we analyzed whether the inhibiting effect on the generation of LAK activity exerted by RBC-30 SN was due to surface down regulation of this activating receptor. As previously reported,[11] the incubation of NK cells with IL-2 induced a strong up regulation of NKG2D receptor (compare the top left with the bottom right quadrant of Fig. 2D). More importantly, the up regulation of NKG2D was strongly affected by NK-cell treatment with RBC-30 SN (Fig. 2D; compare the top right with the bottom left quadrant; MFI, 300 ± 35 in NK cells cultured with IL-2 vs. 75 ± 15 in NK cells cultured with IL-2 and RBC-30 SN, n = 6, $p < 0.001$). On the other hand, RBC-0 and RBC-5 SN did not influence the IL-2–mediated up regulation of NKG2D on NK cells (not shown).

Role of TGF-β1 in RBC-30–mediated regulation of NK-cell cytotoxicity

To determine whether a soluble factor present in RBC-30 SN would be responsible for the inhibition of LAK generation, we analyzed whether anti-TGF-β1 MoAb could counteract the RBC-30 SN-mediated effect. We focus our attention on TGF-β1 as this cytokine was present in detectable amounts in RBC-30 SN (mean, 4.85 ± 1.91 ng; n = 6) compared to lower amounts found in RBC-0 (mean, 0.87 ± 0.6 ng; n = 6) and RBC-5 (mean, 1.23 ± 0.87 ng; n = 6). In addition, we have recently reported that TGF-β1 can inhibit IL-2–induced up regulation of NKG2D surface expression and NK cell–mediated cytolysis.[11] Thus, TGF-β1 may be a suitable candidate as the inhibiting factor present in SN of RBC-30. Interestingly, anti-TGF-β1 MoAb counteracted the effect of RBC-30 SN on the expression of NKG2D surface receptor on NK cells (compare the top right with the bottom left quadrant in Fig. 2C; MFI, 300 ± 35 in NK cultured with IL-2 vs. 75 ± 15 in NK cells cultured with IL-2 and RBC-30 SN; n = 6, $p < 0.001$). Furthermore, we found that the addition of anti-TGF-β1 MoAb at the onset of culture could partially restore the generation of LAK activity inhibited by RBC-30 SN (Fig. 3A).

Role of sHLA-I and sFasL in RBC-30–mediated regulation of NK-cell cytotoxicity

We have recently reported that TGF-β1 can be produced by NK cells upon interaction of specific HLA-I receptors

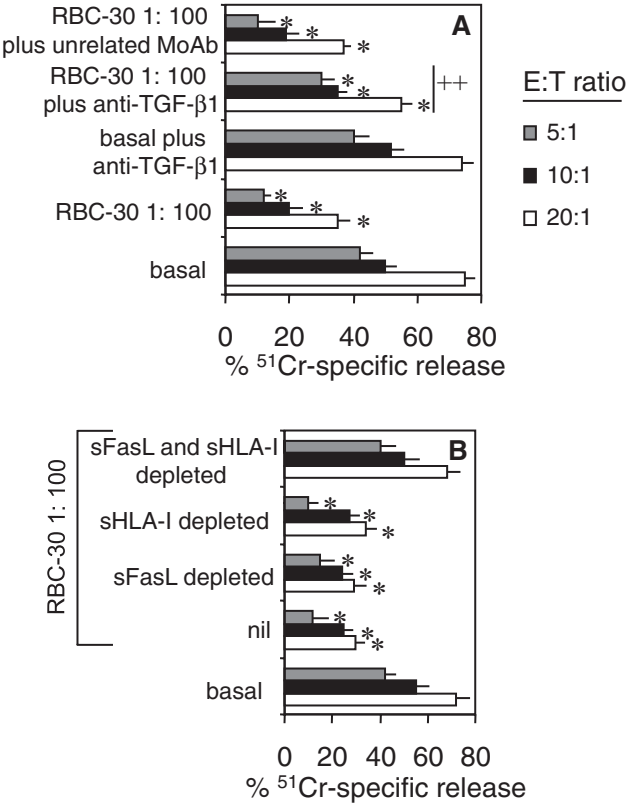

Fig. 3. Role of TGF-β1, sHLA-I, and FasL detectable in RBC-30 SN in inhibiting LAK activity. LAK activity was analyzed using as target cell the LAK-sensitive Jurkat cell line. (A) Cytolysis was analyzed after culture of NK cells with IL-2 for 5 days in culture medium (basal), with RBC-30 SN alone, with anti-TGF-β1 blocking MoAb, or with an unrelated MoAb matched for the isotype. The effect of anti-TGF-β1 MoAb on basal cytolysis is shown for comparison (basal plus anti-TGF-β1). (B) Cytolysis of NK cells cultured with IL-2 for 5 days in medium alone (basal) or with RBC-30 SN depleted by either sHLA-I or sFasL or both. Nil = lysis of NK cells cultured with IL-2 and RBC-30 SN. Results are expressed as percentage of ^{51}Cr-specific lysis and are the mean ± SD of six independent experiments. *$p < 0.001$ versus basal; ++$p < 0.05$ between RBC-30 SN with anti-TGF-β1 MoAb and RBC-30 SN in the absence of MoAb or with an unrelated MoAb matched for isotype. E : T ratios: (■) 5:1; (□) 10:1; (■) 20:1.

and sHLA-I.[11] Upon this interaction, NK cells release sFasL and this sFasL can, in turn, affect NK-cell survival.[18,20] Thus, we have analyzed whether sHLA-I and sFasL could be present in RBC-0, RBC-5 (data not shown), and RBC-30 SN(s). Confirming previously reported data,[12,13] we found a length of storage-dependent increase of both soluble molecules, up to a detectable amount of sHLA-I (mean, 4.42 ± 2.41 μg; n = 6) and sFasL (mean, 20.82 ± 10.3 ng; n = 6) in RBC-30 SN. We then analyzed whether the depletion of sHLA-I and/or sFasL from RBC-30 SN could inhibit the down regulation of LAK generation. Although not

shown, after immunodepletion of sHLA-I and/or sFasL with specific MoAb these factors were almost undetectable in these SNs. As shown in Fig. 3B, RBC-30 SNs depleted of either sHLA-I or sFasL still inhibit LAK effector cell generation. More interestingly, LAK-cell generation was efficient by culturing NK cells with IL-2 and RBC-30 SN depleted of both sHLA-I and sFasL (Fig. 3B).

DISCUSSION

Herein, we show that TGF-β1, sHLA-I, and sFasL are relevant players and appear to be involved in the down regulation of NK cell–mediated cytolysis associated with RBC transfusions. This immunomodulatory effect is evident not only on lysis of NK-sensitive target K562 cell line, but also on activation of NK cells through the engagement of activating receptors as NKG2D, NKp30, and NKp46. These activating molecules have been considered as the main receptors involved in the recognition of different tumor target cells.[10] Thus, this would imply that the regulation induced by blood transfusion might affect several if not most of all tumor target cell lysis. Apparently, the down regulation of NK cell–mediated lysis was not dependent on a reduction of NK cells, identified as CD3– CD56+ lymphocytes, found in peripheral blood after blood transfusion. A possible explanation of the down regulation observed would be that more immature and less cytotoxic NK cells may be recruited in blood stream after transfusion. However, we found that RBC-30 SNs were also able to inhibit the in vitro generation of LAK cells induced by IL-2. The lysis of Jurkat cells, a LAK-sensitive tumor target, mediated mainly by NKG2D was strongly impaired by incubation during LAK cell generation with SN of RBC-30. This effect was partly dependent on the presence of TGF-β1 in these SNs as inactivation of TGF-β1 by specific MoAb partially restored LAK activity. In addition, this restoration was not full, indicating that additional soluble factors should be present in SN of RBC-30. The finding that the depletion of both sHLA-I and FasL could restore completely the generation of LAK activity would suggest that these two factors are responsible for this immunomodulatory effect. According to the hypothesis that TGF-β1, sHLA-I, and sFasL may be involved in down regulation of NK-cell cytolysis, we found that these three factors were present at very low concentrations in SNs derived from blood units after donation or 5 days later. It is difficult to define which among TGF-β1, sHLA-I, and sFasL is the main factor involved in down regulating NK-cell activity. Indeed, we have previously shown that sHLA-I can induce sFasL[12] and TGF-β1[11] secretion by NK cells. In addition, sFasL can induce NK-cell apoptosis while TGF-β1 can block generation of LAK activity.[11] One possible explanation is that sHLA-I is the triggering stimulus able to elicit sFasL and TGF-β1 production, which in turn may affect NK cells. It is to determine whether sFasL can deliver a signal in NK cells that induce TGF-β1 production, besides NK-cell apoptosis.[12] Although not shown, we have found that the oligomerization of Fas receptor with anti-Fas MoAb, mimicking the effect of trimeric sFasL present in RBC-30, can induce activation (i.e., increase in intracellular free calcium concentration) of NK cells. Experiments are in progress to define whether after this activation NK cells can produce TGF-β1.

Whatever the explanation could be, NK cells are implicated in the recognition and destruction of transformed cells as well as in killing of virus-infected cells; thus, the evasion of NK cell–mediated immunosurveillance may be one of the main determinants of cancer development.[6-9] Taking into account the crucial role of NK cells in host immune defense, the herein shown ability of RBC transfusion to induce a marked, although transient, inhibition of NK-cell activity would suggest the possibility that antitumor and antiviral immune response are hampered in transfused patients.

CONFLICT OF INTEREST

All the authors confirm that there is no conflict of interest.

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