Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation

Grazia Maria Spaggiari, Andrea Capobianco, Stelvio Becchetti, Maria Cristina Mingari and Lorenzo Moretta
Mesenchymal stem cell–natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2–induced NK-cell proliferation

Grazia Maria Spaggiari, Andrea Capobianco, Stelvio Becchetti, Maria Cristina Mingari, and Lorenzo Moretta

Introduction

Mesenchymal stem cells (MSCs) are known for their characteristic of being multipotent stem cells, capable of forming bone, cartilage, and other mesenchymal tissues. In particular, in vitro experiments demonstrated that clonal MSCs can differentiate into different lineages including not only osteoblasts, chondrocytes, and adipocytes but also muscle cells, cardiomyocytes, and neural precursors. Moreover, MSCs are a component of the bone marrow stroma that have been shown to support hemopoiesis by providing suitable cytokines and growth factors. More recently, another function has been ascribed to MSCs. Thus, as shown by different groups, MSCs can inhibit T-cell responses induced by mitogens or alloantigens. The mechanisms underlying such immunosuppressive activity are only in part understood. Recent studies suggest that soluble factors produced by MSCs represent key mediators of MSC-mediated inhibition, though cell contact might also be involved.

Little information is available regarding the cellular interactions between natural killer (NK) cells, major effector cells of innate immunity, and MSCs. NK cells are known to display strong cytolytic activity against tumor- or virus-infected cells. Their function is regulated by a series of surface receptors transducing inhibitory or activating signals. Inhibitory receptors are represented mainly by killer immunoglobulin-like receptors (KIRs) that are specific for allotypic determinants shared by different HLA class I alleles and CD94/NKG2A specific for HLA-E (a nonclassic MHC class I molecule), whose levels of expression are directly proportional to HLA class I surface density. In normal conditions, the expression of classic HLA class I or HLA-E molecules on the surfaces of normal autologous cells prevents activation of NK cells because of the interaction with these inhibitory receptors. The down-regulation of HLA class I expression or even the loss of single HLA class I alleles at the surface of tumor- or virus-infected cells can result in a lack of inhibitory interactions and can lead to NK-cell activation. Under these conditions, target cells become susceptible to NK-mediated killing. Different receptors and coreceptors are responsible for NK-cell activation on interaction with target cells. The natural cytotoxicity receptors (NCRs) NKp46, NKp30, and NKp44 represent crucial receptors for NK-cell triggering and mediate cytotoxic activity and cytokine production. Thus far, the NCR ligands expressed on target cells have not been identified. Other important receptors involved in NK-cell activation are NKG2D and DNAM-1, the ligands of which are represented by MICA/B and ULBPs for NKG2D and the poliovirus receptor (PVR) and Nectin-2 for DNAM-1. A series of...
coreceptors capable of supporting NCR-mediated NK-cell triggering has been described. These include 2B4, NTBA, NKP80, and CD59. CD48 represents the known ligand of 2B4, whereas NTBA mediates homophilic interactions.

In this study we analyzed the effect of the interaction between MSC and NK cells. We show that MSCs can inhibit the IL-2–induced proliferation of resting, unactivated NK cells, whereas they had only a partial inhibitory effect on proliferating NK cells. More important, activated NK cells could efficiently lyse MSCs. This information may have relevant implications in novel bone marrow (BM) transplantation approaches in which MSCs are infused to optimize engraftment or to suppress graft-versus-host disease (GVHD).

### Materials and methods

#### Isolation and culture of MSCs

After obtaining ethics committee approval and informed consent, human MSCs were obtained from discarded bone tissues from 15 pediatric patients undergoing surgery to correct major scoliosis. Bone marrow cell suspensions were plated at a concentration of 1 × 10^6 cells/mL in 25-cm^2 tissue-culture flasks in Mesencult basal medium supplemented with MSC stimulatory supplements (both from StemCell Technologies, Vancouver, BC, Canada) and incubated at 37°C in a 5% CO2 humidified atmosphere. After 24 hours, nonadherent cells were removed and fresh medium was added. Half the medium volume was replaced twice a week. When the cultures nearly reached confluence, cells were detached by treatment with trypsin/EDTA solution (BioWhittaker, Cambrex, Verviers, Belgium) and replated at 5 × 10^3 cells per 75-cm^2 tissue-culture flask. MSCs were used in the experiments only after 2 to 3 expansion passages to ensure depletion of CD56^+ NK cells. Alternatively, NK cells were used in cytotoxicity assays immediately after separation or on short-term activation (20 hours–7 days) with 100 U/mL IFN-γ (PeproTech, London, United Kingdom) before their use.

#### Isolation and culture of NK cells

NK cells were isolated from the peripheral blood of healthy donors (some were also donors of MSCs) using the RosetteSep method (StemCell Technologies). Purified NK cells were cultured on irradiated feeder cells in tissue-culture flasks in Mesencult basal medium supplemented with 100 U/mL IL-2 or 2 ng/mL IL-12 (PeproTech).

#### Monoclonal antibodies and cytofluorimetric analysis

The following monoclonal antibodies (mAbs), produced in our laboratory, were used in this study: JT3A (IgG2a, anti-CD3), c127 (IgG1, anti-CD16), c218 (IgG1, anti-CD56), BAB281 and KL247 (IgG1 and IgM, respectively, anti-NKp46), Z231 and K538 (IgG1 and IgM, respectively, anti-NKp44), A76 and F252 (IgG1 and IgM, respectively, anti-NKp30), BAT221 (IgG1, anti-NKG2D), GN18 and F5 (IgG1 and IgM, respectively, anti-DNAM-1), MA127 (IgG1, anti-NTBA), GL183 (IgG1, anti-KIR2DL2/3/52, anti-CD158b1/b2/j), EB66 (IgG1, anti-KIR2DL1/S1, anti-CD158a/b), Z27 (IgG1, anti-KIR3DL1, anti-CD158e), AZ158 (IgG2a, anti-KIR3DL1, anti-CD158e), Z270 and Z199 (IgG1 and IgG2b, respectively, anti-NKG2A), Y9 (IgM, anti-CD94), L95 (IgG1, anti-PVR), L14 (IgG2a, anti-Nectin-2), BAM195 (IgG1, anti-MICA), and A6-136 (IgM, anti–HLA class I).

#### Cytolytic assays

Polyclonal (either short-term or long-term activated) and clonal NK cells were tested for cytolytic activity in a 4-hour chromium 51 (^{51}Cr) release assay, as previously described. Autologous or allogeneic MSCs were used as targets. Briefly, MSCs were detached with trypsin/EDTA (ethylenediaminetetraacetic acid) solution, labeled with 100 μg 51^Cr/10^6 cells, and plated at 5000 cells/well in 96-well V-bottom microplates. To test the lytic potential of NK cells against MSCs, effectors were plated at different effector-to-target (E/T) ratios. For mAb-mediated blocking experiments, NK cells were preincubated with mAbs specific to the various activating or inhibitory receptors (of IgM isotype, if available) at the concentration of 10 μg/mL and, after washing, were used in the cytolytic assay at a 10:1 or 5:1 E/T ratio. For masking of HLA class I molecules, MSCs were preincubated with saturating amounts of the anti–HLA class I A6136 mAb.

To characterize the signaling pattern of activating and inhibitory receptors of polyclonal and clonal NK cells, redirected-killing assays were performed. To this purpose, the murine mastocytoma cell line P815 was used as target in the presence of mAbs of IgG isotype at the concentration of 0.5 μg/mL.
Cytokine production

To evaluate IFN-γ production by NK cells after interaction with MSCs, coculture experiments were performed. Polyclonal activated NK cells were mixed with or without target MSCs in medium with 100 U/mL IL-2 at an E/T ratio of 8:1 in V-bottom, 96-well plates. In some experiments, MSCs were used after 48-hour exposure to IFN-γ. To inhibit cytokine secretion, monensin-containing GolgiStop (Becton Dickinson) was added at the beginning of cocultures. After 5 hours, cells were harvested, and surface and intracellular stainings were performed. Briefly, cells were first labeled with anti-CD56-PE mAb for 20 minutes at 4°C, then washed, fixed, and permeabilized with Cytofix/Cytoperm solution (Becton Dickinson). Afterward, for intracellular cytokine staining, cells were incubated with anti–IFN-γ–PE mAb for 30 minutes at 4°C, then washed and resuspended in PBS 2% FCS for cytofluorimetric analysis.

Results

MSCs prevent the IL-2–induced proliferation of resting NK cells but have only a partial inhibitory effect on proliferating NK cells

MSCs have been shown to sharply suppress T-cell proliferation of resting and activated T cells. To assess whether MSCs could exert a similar inhibitory effect on NK cells, we cultured allogeneic MSCs with resting, unactivated NK cells in the presence of exogenous IL-2 (100 U/mL) or with proliferating NK cells that had been cultured in IL-2 for more than 7 days. As shown in Figure 1A, MSCs prevented the proliferation of resting NK cells. It is of note that the inhibition of NK-cell proliferation occurred at all NK/MSC ratios tested (ranging from 1:1 to 8:1; data not shown). On the other hand, as shown in Figure 1B, only partial inhibition of proliferation of activated NK cells could be detected, and the extent of the inhibitory effect was dependent on the NK/MSC ratio. We further evaluated whether MSCs could also inhibit NK-cell proliferation induced by IL-15. Although not shown, MSCs had a similar suppressive effect on fresh NK cells when cultured in the presence of 100 ng/mL IL-15.

Figure 1. MSC-induced inhibition of NK-cell proliferation. NK cells were cultured alone or with allogeneic irradiated MSCs in the presence of 100 U/mL IL-2. (A) Results of 1 of 8 representative experiments in which the proliferation of resting NK cells was evaluated using the CFSE dilution method. CFSE fluorescence of NK cells (identified as CD45−CD56+ lymphocytes) was analyzed after 7 days of culture alone or with MSCs (NK/MSC ratio, 8:1). (B) Proliferative response of IL-2–cultured proliferating NK cells analyzed at day 7 of culture in the absence or presence of MSCs. Data are presented as percentage of 3H-thymidine incorporation by NK cells cultured in the presence of MSCs (at different NK/MSC ratio) with respect to NK cells cultured alone (100%). Error bars represent mean ± SD of 6 independent experiments.

Table 1. Surface expression of the ligands of DNAM-1 and NKG2D activating receptors and of HLA class I molecules in different MSC cell populations

<table>
<thead>
<tr>
<th>Donor</th>
<th>DNAM-1 ligands</th>
<th>NKG2D ligands</th>
<th>HLA class I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nectin-2 MRFI*</td>
<td>PVR MRFI</td>
<td>MICA/B and ULBPs† (MRFI) MRFI</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>24</td>
<td>ULBP3 (3) 69</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>16</td>
<td>ULBP3 (3) 31</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8</td>
<td>ULBP3 (2) 10</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>21</td>
<td>ULBP3 (2) 50</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>35</td>
<td>ULBP1 (7), 2 (2), 3 (2), 4 (3) 47</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>16</td>
<td>ULBP1 (2), 2 (3), 3 (4), 4 (3) 40</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>33</td>
<td>ULBP2 (3), 3 (4) 61</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>18</td>
<td>MICA (2), ULBP1 (2), 2 (2), 3 (2) 25</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>6</td>
<td>MICA (2), ULBP2 (2), 3 (2) 8</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>17</td>
<td>MICA (2), ULBP1 (2), 3 (2) 17</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>10</td>
<td>ULBP1 (2), 3 (2) 20</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>6</td>
<td>ULBP3 (2) 14</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>15</td>
<td>MICA (2), ULBP3 (3) 30</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>12</td>
<td>ULBP3 (2) 14</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>10</td>
<td>MICA (2), ULBP3 (3) 38</td>
</tr>
</tbody>
</table>

*Concerning MRFI, see “Materials and methods.”
†Only the expressed molecules among MICA and ULBP1-4 are indicated.

Surface expression on MSCs of ligands recognized by different activating NK receptors

To analyze possible interactions between NK cells and MSCs, we first assessed MSCs for the expression of ligands recognized by activating NK receptors. We performed cytofluorimetric analysis using mAbs specific for known ligands of activating receptors and checked, in parallel, the presence of typical markers of MSCs. Table 1 shows the level of expression of the ligands of DNAM-1 and NKG2D triggering receptors and of HLA class I molecules in the 15 MSC populations analyzed in this study. Remarkably, MSCs expressed PVR and Nectin-2 (both ligands of DNAM-1) as well as ULBPs and MICA (both ligands of NKG2D). In particular, among the ligands of NKG2D, ULBP3 was expressed in all 15 MSC populations analyzed. Moreover, in no instance were CD48 (ligand of 2B4) and NTBA (mediating homophilic interactions) molecules expressed by MSCs. In agreement with previous reports, MSCs did not express CD34, CD45, or HLA-DR (data not shown), whereas they did express low/intermediate levels of HLA class I molecules. Figure 2 shows the cytofluorimetric analysis of MSCs derived from a representative donor (donor 2). Figure 2A shows the expression profiles of CD105, CD166, and CD29 (typical MSC markers). Figure 2B shows the surface expression of ULBP3, Nectin-2, and PVR molecules.

IL-2–activated, but not resting, NK cells lyse MSCs

The finding that MSCs expressed ligands for activating NK receptors, together with the low expression of HLA class I molecules, suggested the possibility that MSCs could be susceptible to NK cell–mediated lysis. To test this hypothesis, we analyzed freshly isolated NK cells and NK-cell populations that had been exposed to IL-2 for a short (20-hour) or a long (7-day) interval for their ability to kill allogeneic MSC target cells. In agreement with a previous report, freshly isolated NK cells did not lyse MSCs, even when used at high E/T ratios (Figure 3A-C). However, the same NK cells, when exposed to IL-2 for 20 hours only, displayed strong cytolytic activity. For example, approximately 50% specific lysis was achieved at a 6:1 NK/MSC ratio. NK cells cultured in IL-2 for 7 days displayed even higher cytolytic...
activity. Although not shown, short-term culture of freshly isolated NK cells in the presence of IL-12 did not induce any significant cytolysis of MSCs, whereas low cytolytic activity could be detected only after 72 hours of culture. Having demonstrated the susceptibility of MSCs to lysis of allogeneic effector NK cells, we asked whether lysis also occurred in an autologous setting. To this aim, we performed a series of cytotoxicity experiments in which different MSC populations were used as target and autologous or allogeneic NK cells were used as effector. It is of note that these experiments were performed using long-term IL-2–activated NK cells because of the low numbers of NK cells isolated from the blood samples of pediatric donors. As shown in Figure 3D-F, MSCs were susceptible to NK-mediated lysis independently of whether autologous or allogeneic NK cells were used.

Analysis of the activating or inhibitory molecular interactions involved in the lysis of autologous and allogeneic MSCs

To define which receptor–ligand interactions were involved in the NK-mediated killing of MSCs, we used the mAb-mediated masking strategy in cytotoxicity assays. Figure 4 shows the results of 2 representative experiments. In Figure 4A, NK cells derived from an allogeneic donor were used as effector cells against $^{51}$Cr-labeled MSCs. mAb-mediated masking of NKp30, NKG2D, and DNAM-1 resulted in the inhibition of lysis. On the other hand, blocking of Nkp46 resulted in low inhibition, whereas Nkp44 did not appear to exert any substantial role. Consistent results were obtained in 10 independent experiments using different MSCs and different allogeneic NK-cell populations. Figure 4B shows a cytotoxicity assay in which NK cells were tested against autologous MSCs. In addition, in this autologous setting, the major activating NK receptors involved were NKp30, NKG2D, and DNAM-1. Similar results were obtained in 5 independent experiments using autologous combinations. Notably, in allogeneic and autologous NK/MSC combinations, the simultaneous masking of these receptors virtually abrogated cell lysis. Remarkably, autologous MSCs are as susceptible to lysis as allogeneic MSCs. These data suggest that interactions involving HLA class I–specific inhibitory receptors, possibly occurring between NK cells and MSCs, were not sufficient to protect MSCs from lysis. In fact, mAb-mediated masking of HLA class I molecules on target cells did not result in any significant increase of lysis (Figure 4). This is in agreement with the low levels of HLA class I molecules on the surface of MSCs. In addition, mAb-mediated blocking of the HLA-E–specific inhibitory receptor CD94/NKG2A did not result in increased cytotoxic activity. Given that the levels of surface HLA-E usually parallel the HLA class I surface density, our results suggest that MSCs are characterized by low or absent expression of HLA-E molecules.

Decreased susceptibility of MSCs to NK-cell–mediated lysis after exposure to IFN-γ: role of HLA class I molecules

During infection or, more generally, during inflammatory responses, cells are exposed to proinflammatory cytokines, such as IFN-γ. IFN-γ has been shown to up-regulate the surface density of HLA class I and HLA class II molecules on MSCs. To test whether IFN-γ could modify the surface expression of ligands recognized by inhibitory or activating NK receptors (with a possible effect on the susceptibility of MSCs to NK-cell–mediated lysis of MSCs.
lysis), we cultured 5 different MSC populations with IFN-γ (100 U/mL). After 48 hours, IFN-γ-treated or untreated MSCs were analyzed by cytfluorometry to assess the expression of informative surface molecules. In agreement with previous data, IFN-γ induced increases in the levels of HLA class I (Figure 5A) and HLA class II (not shown) molecules in all MSC populations tested. Moreover, the expression of ICAM-1 was augmented in IFN-γ–treated MSCs (not shown). On the other hand, IFN-γ treatment did not significantly modify the expression of adhesion molecules (with the exception of ICAM-1). Interestingly, PVR and Nectin-2—the ligands of DNAM-1—but not those of NKG2D (such as ULBP3), were up-regulated (Figure 5A). We next tested the susceptibility of IFN-γ–treated MSCs to NK cell–mediated cytotoxic activity. MSCs were found to be less susceptible to lysis. Figure 5B shows a representative cytotoxicity assay in which NK cells were used against autologous MSCs that had been cultured in the presence of IFN-γ or in medium alone. Polyclonal NK cells efficiently lysed untreated MSCs at a 10:1 E/T ratio. Monoclonal antibody–mediated masking of HLA class I molecules or CD94/NKG2A receptor could not induce increases in lysis. On the other hand, the same NK cells were poorly cytolytic against IFN-γ–treated MSCs. In this case, mAb-mediated blocking of HLA class I or CD94/NKG2A resulted in sharp increases of cytotoxicity. These results suggest that not only classic HLA class I but also HLA-E molecules on the surfaces of MSCs can inhibit not only NK-cell lysis, but also NK-cell–mediated cytotoxic activity. MSCs are susceptible to NK-mediated lysis and that different NK receptor–ligand interactions contribute to NK-cell activation and MSC lysis. In addition, proliferative responses of resting NK cells to IL-2 or IL-15 were blocked even by low numbers of MSCs. In the case of activated NK cells, only a partial decrease in proliferative capacity could be detected. The mechanism(s) by which MSCs would inhibit NK-cell proliferation has not yet been identified. It is conceivable that inhibition may reflect the production of soluble factors, as has been shown for MSC–T-cell interaction. The role of TGF-β, IL-10, PGE2, and IDO are being investigated in our laboratory. A previous study reported that MSCs were resistant to alloreactive CTLs capable of lysing PHA-blasts from the same MSC donor.43 This finding is compatible with the low surface expression of HLA class I molecules. On the other hand, the surface expression of low levels of HLA class I molecules favors the NK-mediated lysis of MSCs, as described in the present study.

Discussion

Our data provide information on the result of the interaction between NK cells and MSCs. We show that MSCs are susceptible to NK-mediated lysis and that different NK receptor–ligand interactions contribute to NK-cell activation and MSC lysis. In addition, proliferative responses of resting NK cells to IL-2 or IL-15 were blocked even by low numbers of MSCs. In the case of activated NK cells, only a partial decrease in proliferative capacity could be detected. The mechanism(s) by which MSCs would inhibit NK-cell proliferation has not yet been identified. It is conceivable that inhibition may reflect the production of soluble factors, as has been shown for MSC–T-cell interaction. The role of TGF-β, IL-10, PGE2, and IDO are being investigated in our laboratory. A previous study reported that MSCs were resistant to alloreactive CTLs capable of lysing PHA-blasts from the same MSC donor.43 This finding is compatible with the low surface expression of HLA class I molecules. On the other hand, the surface expression of low levels of HLA class I molecules favors the NK-mediated lysis of MSCs, as described in the present study.
Moreover, MSCs expressed different ligands recognized by activating NK receptors, including PVR and Nectin-2 (DNAM-1 ligands) and ULBPs (NKG2D ligands). Accordingly, mAb-mediated blocking of NKG2D and DNAM-1 resulted in the partial inhibition of lysis. In addition, receptor-blocking experiments using specific mAbs revealed that lysis of MSCs was also dependent on NKP30 and NKP46, though the cellular ligands of these receptors have not been identified so far. Remarkably, NK cells could lyse not only allogeneic but also autologous MSCs. This finding is reminiscent of previous data regarding the susceptibility of immature DC (iDC) to lysis by autologous NK cells.46 NK-mediated lysis of iDC is thought to represent a mechanism of quality control, allowing positive selection of those DCs undergoing full maturation and thus capable of promoting optimal T-cell priming.37,48 In the case of iDC, however, NK-cell activation involved primarily the NKP30 receptor, whereas the lysis of MSCs is mediated by different receptor–ligand interactions. The fact that NK cells can lyse autologous MSCs may prompt one to ask why MSCs are not killed by NK cells in vivo. This may reflect differences between MSCs cultured in vitro and those existing in vivo. The latter represent an infrequent cell type that could perhaps be localized in niches, thus escaping the NK-mediated attack, or could express higher levels of HLA class I molecules, or lack ligands for activating receptors. This would render MSCs resistant to NK-mediated killing. Moreover, in vivo NK cells might not reach, under normal conditions, an activation state sufficient for killing MSCs. Whatever the explanation, cytolytic interactions between NK cells and MSCs might occur with BM transplantation in which cytokines capable of inducing NK-cell activation can be released. Indeed, our present data clearly show that even short-term exposure to IL-2 can induce strong NK cytotoxicity against MSCs. Thus, our data suggest that, in an inflammatory environment, NK cells may become potentially capable of lysing MSCs. Notably, MSCs are the precursors of stromal BM cells and are thought to favor hematopoietic-cell engraftment while preventing GVHD.39 Accordingly, MSCs, obtained under culture conditions similar to that of the present study, have been used in experimental protocols together with CD34+ hematopoietic-cell precursors.39,40 However, under these conditions, NK cells generated from donor CD34+ cells beyond 2 to 3 weeks after transplantation should not cause any relevant damage to MSCs because, during the same time interval, the latter might have undergone differentiation to BM stromal cells and other tissues. The adoptive transfer of NK cells has been proposed in the therapy of acute myeloid leukemias in allogeneic bone marrow transplantation to eradicate leukemic cells.50-52 The adoptive infusion of activated NK cells could potentially kill MSCs if they are infused shortly before or simultaneously with NK cells. Note, however, that this effect could be, at least in part, counteracted by the effect of IFN-γ. Indeed this cytokine, which is released by NK cells and other cell types, induced the up-regulation of HLA class I molecules in MSCs, thus rendering these cells resistant to NK-mediated lysis. It would be of interest to analyze the final outcome of the in vivo interactions between NK cells and MSCs in suitable murine models, in experimental settings reproducing conditions that occur in BM transplantation–associated adoptive immunotherapy involving donor NK cells and MSCs.

In conclusion, our results provide relevant information on the possible effect of NK cells on MSCs. Whether these interactions are relevant in physiologic conditions remains to be determined; however, our data offer an interesting clue regarding possible interferences occurring when the two cell types are used in combination in approaches of adoptive immunotherapy.

References

7. Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP, Gerson SL. Human marrow-derived stromal BM cells and are thought to favor hematopoietic-cell engraftment while preventing GVHD. Accordingly, MSCs, obtained under culture conditions similar to that of the present study, have been used in experimental protocols together with CD34+ hematopoietic-cell precursors. However, under these conditions, NK cells generated from donor CD34+ cells beyond 2 to 3 weeks after transplantation should not cause any relevant damage to MSCs because, during the same time interval, the latter might have undergone differentiation to BM stromal cells and other tissues. The adoptive transfer of NK cells has been proposed in the therapy of acute myeloid leukemias in allogeneic bone marrow transplantation to eradicate leukemic cells. The adoptive infusion of activated NK cells could potentially kill MSCs if they are infused shortly before or simultaneously with NK cells. Note, however, that this effect could be, at least in part, counteracted by the effect of IFN-γ. Indeed this cytokine, which is released by NK cells and other cell types, induced the up-regulation of HLA class I molecules in MSCs, thus rendering these cells resistant to NK-mediated lysis. It would be of interest to analyze the final outcome of the in vivo interactions between NK cells and MSCs in suitable murine models, in experimental settings reproducing conditions that occur in BM transplantation–associated adoptive immunotherapy involving donor NK cells and MSCs.

In conclusion, our results provide relevant information on the possible effect of NK cells on MSCs. Whether these interactions are relevant in physiologic conditions remains to be determined; however, our data offer an interesting clue regarding possible interferences occurring when the two cell types are used in combination in approaches of adoptive immunotherapy.


