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Immunoregulatory Properties of Mouse Limbal Stem Cells

Vladimir Holan,*† Katerina Pokorna,*† Jana Prochazkova,*† Magdalena Krulova,*† and Alena Zajicova*

Stem cells have been demonstrated in nearly all adult mammalian tissues and play a vital role in their physiological renewal and healing after injury. Due to their irreplaceable role in tissue repair, these cells had to develop mechanisms protecting them from deleterious inflammatory immune reactions and ensuring their increased resistance to various apoptosis-inducing agents. In this study, we demonstrate that a population of mouse limbal cells highly enriched for cells expressing markers and characteristics of limbal stem cells (LSCs) suppresses in a dose-dependent manner the proliferation of lymphocytes elicited by mitogens or TCR-triggering and significantly inhibits the production of proinflammatory cytokines by activated T cells. The suppression was mediated by soluble factor(s) and did not affect early cell activation. LSCs were even more suppressive than mesenchymal stem cells or natural regulatory T cells. In addition, the cells expressing markers and characteristics of LSC had significantly higher levels of mRNA for Fas ligand and for the antiapoptotic molecules Mcl-1, XIAP, and survivin than other limbal cell populations. LSCs were also more resistant to staurosporin-induced apoptotic cell death and to cell-mediated cytotoxic reaction than other limbal cells. Collectively, these results suggest that SC isolated from fresh adult limbal tissue possess immunomodulatory properties and inhibit proinflammatory immune reactions. Simultaneously, these cells express high levels of mRNA for antiapoptotic molecules, which can protect them against cell-mediated cytotoxic reactions and various apoptosis-inducing agents. The Journal of Immunology, 2010, 184: 2124–2129.

Adult tissue specific stem cells (SCs) represent a diverse group of multipotent SCs clustered in various niches throughout the body and serve as a vital renewable source of specialized cells for tissue development, maintenance, and repair (1–3). In many specialized organs, such as the cornea, the damage of a particular source of SC can result in an irreversible loss of function of the tissue or organ and ultimately, in the case of the eye, in blindness (4, 5). Such a deficiency in limbal stem cells (LSCs) may be congenital or caused by mechanical injury, various toxic substances, or harmful local inflammatory reactions. To protect SCs from deleterious immune reactions, the cells themselves or their niches should possess immunomodulatory properties.

Indeed, the immunosuppressive properties of mesenchymal stem cells (MSCs) have been documented. It has been shown that bone marrow-derived MSCs inhibit lymphocyte proliferation in vitro (6, 7), the production of cytokines (8), the formation of cytotoxic CD8+ T lymphocytes (9), and immune responses in vivo (10–13). It has been proposed that MSCs can contribute to the control of inflammatory diseases, as has been demonstrated by the MSC-mediated attenuation of inflammation in myocarditis (14), rheumatoid arthritis (15), and experimental autoimmune diseases (16, 17). After the transfer of MSCs onto the damaged ocular surface, the cells support epithelial healing (18); this effect has been attributed to the suppression of local inflammatory reactions (19). In addition, MSCs themselves are relatively resistant to CTL-mediated lysis (20).

Cells with the characteristics of MSCs represent a heterogenous population of SCs that have been isolated from almost every type of tissue stroma where they share their principal immunomodulatory and regenerative properties. These cells are distinct from adult tissue specific SCs, which express specific SC markers, have different growth properties, and exhibit the side-population (SP) phenotype. Although the immunosuppressive properties of MSCs have been well documented, the immunoregulatory properties of adult tissue specific SCs have not been described. In this study, we show that SC-mediated immnosuppression is a more general phenomenon and not only confined to MSC. Using a recently described procedure (21), we isolated from the limbal tissue of adult mice a small population of cells with LSC markers and characteristics, and we show that these cells effectively inhibit lymphocyte proliferation and modulate cytokine production. In addition, LSCs display an enhanced expression of genes for the antiapoptotic proteins Mcl-1, XIAP, and survivin and are more resistant to CTL-mediated or apoptotic cell death than other limbal cells. We suggest that adult tissue specific SCs have immunosuppressive properties and possess mechanisms protecting them against various deleterious immunological and apoptosis-inducing reactions, thus supporting their survival in the body.

Materials and Methods

Mice

BALB/c mice of both sexes were obtained at the age of 8–12 wk from the breeding unit of the Institute of Molecular Genetics, Prague, Czech Republic. The use of animals was approved by the local Animal Ethics Committee.

LSC isolation

A population of cells expressing markers and characteristics of LSC was isolated from trypsin digested limbal tissue, as recently described (21). In brief, limbal tissues from 15–20 BALB/c mice were subjected to 10 short
cycles of trypsin digestion, and the obtained single cell suspensions were separated by centrifugation (10 min, 300 × g) on a discontinuous Percoll gradient. One small cell fraction, obtained on an 80% Percoll gradient (representing ~5% of the total limbal cells), exhibited markers (expression of transporter protein ABCG2 and transcription factor p63, the absence of cytokeratin K12 and connexin 43) and characteristics (small dense cells, exhibition of the SP phenotype, very low spontaneous proliferation, significant proliferation on appropriate feeder cells) of LSC. This fraction of limbal cells was used as a source of LSCs. Unseparated limbal cells or the cells from the fraction obtained on a 50% Percoll gradient were used as control cells.

Isolation of MSCs and natural regulatory T cells

MSCs were isolated from the bone marrow of BALB/c mice. The bone marrow from the femurs and tibia was flushed out, washed, and cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% FCS (Sigma-Aldrich), antibiotics (100 μg/ml of streptomycin, 100 U/ml of penicillin), 1% nonessential amino acids, and 10% FCS (hereafter referred to as complete RPMI 1640 medium). The cells were cultured at a concentration of 4 × 10^6 cells/ml in 5 ml complete RPMI 1640 medium in 25 cm² tissue culture flasks (Nunc, Roskilde, Denmark). After a 24-h incubation period, the nonadherent cells were removed by washing, and the remaining adherent cells were cultured for at least 3 wk with regular exchange of the culture medium and passaging of the cells to maintain an optimal concentration. The phenotype of the cells was determined by flow cytometry. Over 90% of the growing cells were negative for MHC class II molecules, CD86, and CD11c, whereas the majority of them expressed CD105 and were able to differentiate into adipocytes (data not shown).

Natural regulatory T cells (Tregs) were isolated from the spleens of BALB/c mice by using a CD4^+CD25^+ Regulatory T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and an AutoMACS magnetic separation system (Miltenyi Biotec), as previously described (22). FACS analysis of the sorted cells showed that over 95% of the cells expressed the Treg phenotype, characterized by the expression of CD4, CD25, and forkhead Foxp3.

Cell proliferation and suppression assays

Spleen cells (0.5 × 10^6/ml) from BALB/c mice were stimulated in a volume of 0.2 ml complete RPMI 1640 medium in 96-well tissue culture plates (Corning Co., Corning, NY) with 10 μg/ml mAb anti-CD3 (23), Con A (1.0 μg/ml, Sigma-Aldrich) or LPS (1.0 μg/ml, Difco Laboratories, Detroit, MI). Cell proliferation was determined by adding [3H]thymidine (0.5 Ci/well, Nuclear Research Institute, Rez, Czech Republic) for the last 6 h of the 72-h incubation period. To assess the cell proliferation, unseparated limbal cells or cells from individual limbal cell fractions, MSCs, or Tregs were added to these cultures at the indicated ratio (ranging from 1: 2–1: 32), and cell proliferation was determined. In some experiments, limbal cells were separated from stimulated spleen cells in tissue culture inserts with a 0.2 μm membrane (Nunc, or supernatants were prepared by a 48-h incubation of unseparated limbal cells or cells from 50% or 80% limbal cell fractions (0.4 × 10^6 cells/ml) and were added to the cultures of spleen cells stimulated with mAb anti-CD3 to yield a final concentration of 30%.

To characterize the suppressive molecule, neutralization mAb anti–IL-10 (clone JES5, BioLegends, San Diego, CA) or anti–TGF-β1 (clone 1D11, BioLegend, San Diego, CA) were used for 48-h incubation of unseparated limbal cells or spleen cells with irradiated BALB/c spleen cells) at an effecter-to-target ratio of 5:1. The percentage of specific cytotoxicity was calculated according to the formula:

\[ \text{cytotoxicity} = \left( \frac{\text{maximum release} - \text{spontaneous release}}{\text{maximum release}} \right) \times 100 \]

Determination of the susceptibility of LSC to cell-mediated cytotoxicity

Unseparated limbal cells or cells from limbal cell fractions obtained on 50% or 80% Percoll gradients were labeled with [31Cr] (Amersham Biosciences, Uppsala, Sweden; 100 μCi [31Cr]/2 × 10^5 cells) and cultured in RPMI 1640 medium for 45 min, then washed three times in culture medium. [31Cr]-labeled Con A-induced blasts prepared by stimulation (1 μg Con A/ml) from lymph node cells in BALB/c mice were used as a positive target cell control. The labeled cells (1 × 10^5) were incubated in 96-well tissue culture plates (Nunc) in a volume of 0.2 ml complete RPMI 1640 medium with lymphocytes in standard 90% of 0.4 ml complete RPMI 1640 medium in 48-well tissue culture plates (Corning Co., Corning, NY) with 10^5/ml Con A (clone 4A4, BioLegend, San Diego, CA). Data were collected using a LSRII cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Tree Star, Ashland, OR).

Detection of gene expression by real-time PCR

Total RNA was extracted from unseparated limbal cells or cells of individual limbal cell fractions obtained on the Percoll gradient using TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s instructions. Two μg total RNA was treated using DNase I (Sigma-Aldrich) and used for subsequent reverse transcription. The first-strand cDNA was synthesized using random hexamers (Promega, Madison, WI) in a total reaction volume of 25 μl using M-MLV Reverse Transcriptase (Promega). Quantitative real-time RT-PCR was performed on the iCycler (Bio-Rad, Hercules, CA). The relative quantification model with efficiency correction was applied to calculate the expression of the target gene in comparison with GAPDH used as the housekeeping gene. The primers used for amplification were: Mcl-1: 5'-CTGGTGTGCGCGTCTTTAG-3' (sense), 5'-TGACCACTTTGCTGTCTACTTG-3' (antisense); XIAP: 5'-GCTTCAAGAGCTGAGTATT-3' (sense), 5'-TGGTCTACATCGTGAG-3' (antisense); survivin: 5'-TGATTGGGCCAGTGTGTTT-3' (sense), 5'-CAGGGGACTGCTTCTATGC-3' (antisense); GAPDH: 5'-AGAACCATACCTCCGATC-3' (sense), 5'-ACATTGGGGTAGAGAC-3' (antisense); and Fas ligand (FasL): 5'-ACCGGTGTATTTCAATG-3' (antisense), 5'-AGGCTTTGGTGTGAACACT-3' (sense). The PCR parameters for 25 μl reactions included denaturation at 95°C for 3 min, then 40 cycles at 94°C for 10 s, annealing at 60°C for 20 s and elongation at 72°C for 20 s. iQ SYBR Green Supermix (Bio-Rad) was used for all experiments. Each single experiment was done in triplicate, and the reaction efficiency for each gene was estimated by the dilution curve method. Fluorescence data were collected at each cycle after an elongation step at 80°C for 5 s, and the data were analyzed on the iCycler Detection System, Version 3.1 (Bio-Rad).

Determination of the susceptibility of LSC to cell-mediated cytotoxicity

Because the number of available fresh LSCs was too low to measure apoptosis by flow cytometry, we determined cell death induced by apoptosis-inducing reagent staurosporin using a [31Cr] release assay. Unseparated limbal cells or cells from Percoll fractions were labeled with [31Cr] and incubated for 4 h alone or in the presence of 1 μM staurosporin (Sigma-Aldrich). The percentage of staurosporin-induced cell death for individual cell populations was determined according to the formula:

\[ \% \text{cytotoxicity} = \left( \frac{\text{maximum release} - \text{spontaneous release}}{\text{maximum release}} \right) \times 100 \]

Statistical analysis

The data showed a normal distribution, and the results are expressed as mean ± SD. Comparisons between two groups were analyzed by Student t test, and multiple comparisons were analyzed by ANOVA followed by the Bonferroni post hoc test. A value of p < 0.05 was considered statistically significant.
Table 1. The characteristics of limbal cell populations obtained on Percoll gradients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Unseparated Limbal Cells</th>
<th>Fraction 50%</th>
<th>Fraction 80%</th>
</tr>
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<tbody>
<tr>
<td>Expression of ABCG2</td>
<td>++</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>p63</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K12</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Connexin 43</td>
<td>+</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>SP phenotype</td>
<td>++</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Spontaneous proliferation</td>
<td>+</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Proliferation on feeder</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>cells</td>
<td>Heterogenous</td>
<td>Large</td>
<td>Small</td>
</tr>
</tbody>
</table>

–, no expression; +, low expression; ++, middle expression; ++++, high expression.

Results

Characterization of the LSC population

Limbal tissues from a group of 20 BALB/c mice were enzymatically dissociated, and the cells were separated on a Percoll gradient as recently described (21). One of the cell fractions, obtained on an 80% Percoll gradient and representing ~3–5% of total limbal cells, was highly enriched for cells expressing markers and characteristics of LSC (Table 1). This fraction contained small dense cells that express the putative LSC markers ABCG2 and p63 and were negative for the cornea-associated markers K12 and connexin 43. A significant number of these cells exhibited the SP phenotype and were in the quiescent state. These markers and characteristics of LSC were absent or only barely detectable in other limbal cell fractions. Unseparated limbal cells or cells of the 50% Percoll fraction were used as control cells.

LSC inhibit lymphocyte proliferation

Spleen cells were stimulated through the TCR (anti-CD3) or by a T (Con A) or B (LPS) cell mitogen in the presence of LSC or other limbal cell populations (the ratio of spleen to limbal cells was 8:1), and cell proliferation was determined. As demonstrated in Fig. 1, cell proliferation triggered by TCR stimulation (Fig. 1A) or by T (Fig. 1B) or B (Fig. 1C) cell mitogens was significantly

(p < 0.001) inhibited in the presence of LSCs, but not in the presence of unseparated limbal cells or cells from the cell fraction obtained on a 50% Percoll gradient.

To assess whether the suppression requires cell-to-cell contact or is mediated by cell-free factors, limbal cells were separated from spleen cells in tissue culture inserts with a 0.2 μm membrane. As shown in Fig. 1D, the separation of LSCs from spleen cells by a semipermeable membrane did not abrogate the suppression. In addition, supernatants were prepared by a 48-h incubation of LSC or unseparated limbal cells or cells from 50% Percoll fractions. These supernatants were added to the cultures of anti-CD3–stimulated spleen cells, and cell proliferation was determined. The results showed that the supernatants from the LSCs (the fraction from the 80% Percoll gradient), but not from the control cells, significantly inhibited proliferation of spleen cells (Fig. 1E).

To characterize the suppressive molecule, neutralization mAb anti–IL-10 or anti–TGF-β, AMT, or indomethacine was added to the cultures of spleen cells stimulated with mAb anti-CD3 in the presence of LSCs. As demonstrated in Fig. 1F, neither mAb anti–IL-10, anti–TGF-β, nor the inhibitors used significantly abrogated the LSC-mediated suppression of cell proliferation.

Comparison of suppressor activity of LSCs, MSCs, and Tregs

Spleen cells were stimulated with mAb anti-CD3 in the presence of LSCs, MSCs, or Tregs at the indicated cell ratios. As demonstrated in Fig. 2, all tested cell types significantly inhibited T cell proliferation, but only LSC retained suppressor activity even at a lower concentration (at a ratio of 1:16), when Tregs or MSC were no longer suppressive.

Suppression of cytokine production by LSCs

Spleen cells were stimulated with Con A in the presence of LSCs or unseparated limbal cells or cells from the cell fraction obtained on a 50% Percoll gradient (the ratio of lymphocytes to limbal cells was 8:1), and the presence of IL-2, IL-6, and IFN-γ in the supernatants was determined by ELISA. As shown in Fig. 3, the production of all tested cytokines was significantly inhibited in the presence of LSC, but not in the presence of control cells.

![FIGURE 1. Suppression of lymphocyte proliferation by LSC. Spleen cells (0.5 × 10⁶/ml) from BALB/c mice were cultured unstimulated (--) or stimulated with 10 μg/ml mAb anti-CD3 (A), Con A (1 μg/ml) (B), or LPS (1.0 μg/ml) (C) in the absence or presence (at a ratio of 8:1) of unseparated limbal cells (Uns.) or cells from 50% (50) or 80% Percoll (i.e., LSC) gradient fractions. D, LSCs (fraction 80%) were separated from spleen cells in inserts with a 0.2 μm membrane. E, The supernatants obtained after the culturing of limbal cells were added (30% v/v) to cultures of spleen cells stimulated with mAb anti-CD3. F, Neutralization mAb anti–IL-10, anti–TGF-β1, AMT, or indomethacine (Indo) was added to the cultures of spleen cells stimulated with mAb anti-CD3 in the presence of LSC. Cell proliferation was determined by [3H]thymidine added to the cultures for the last 6 h of the 72-h incubation period. Each bar represents the mean ± SD from three to four independent experiments; each experiment was based on a group of 20 BALB/c mice used for LSC isolation. Asterisks indicate a statistically significant difference (p < 0.01) from the positive control.](http://www.jimmunol.org/DownloadedFrom)
FIGURE 2. Comparison of the suppressor activity of LSCs, MSCs, and Tregs. Spleen cells (0.5 \times 10^6/ml) were cultured unstimulated (−) or stimulated with 10 \mu g/ml anti-CD3 in the absence or in the presence of LSCs, MSCs, or Tregs at the indicated spleen cell to added cell ratio. Cell proliferation was determined by [3H]thymidine added to the cultures for the last 6 h of the 72-h incubation period. Each bar represents the mean ± SD from three to four independent experiments. Values with asterisk are significantly different (p < 0.01) from MSCs or Tregs.

Effect of LSCs on the expression of early cell activation markers

To test the effects of LSCs on the expression of the early cell activation markers CD25 and CD69, spleen cells were stimulated with Con A in the presence of LSC (at a ratio of 8:1). As demonstrated in Fig. 4, the stimulation of spleen cells significantly enhanced the expression of CD25 (Fig. 4A) and CD69 (Fig. 4B) on CD4+ cells, but the expression was not inhibited in the presence of LSC.

The expression of genes for FasL and the antiapoptotic molecules Mcl-1, XIAP, and survivin on LSCs

The expression of genes for FasL that can induce the apoptotic death of activated T cells and for the antiapoptotic molecules Mcl-1, XIAP, and survivin was determined by real-time PCR in unseparated limbal cells and in cells from fractions from 50% and 80% Percoll gradients. As demonstrated in Fig. 5, the expression of the genes for FasL, survivin, Mcl-1, and XIAP was significantly higher in the LSC population than in unseparated limbal cells or in cells from a 50% Percoll gradient. The expression of genes for two other tested antiapoptotic molecules, Bcl-2 and Bcl-XL, was comparable in LSCs and unseparated limbal cells (data not shown).

Resistance of LSC to staurosporin-induced or CTL-mediated cell death

Staurosporin is a reagent commonly used to induce apoptotic cell death in various cell types. Because we had limited numbers of LSCs, which did not allow the use of flow cytometry to analyze staurosporin-induced apoptosis by determining Annexin, we quantified staurosporin-induced cell death by the method of [51Cr] release from labeled cells. Unseparated limbal cells or cells from 50% or 80% Percoll fractions were labeled with [51Cr] and incubated for 4 h with 1 \mu M staurosporin. Significantly less killing was observed in the fraction of LSC (Fig. 6A).

To compare the resistance of limbal cell fractions to cell-mediated cytotoxicity, unseparated limbal cells or cells from 50% or 80% Percoll fraction (or Con A-induced cell blasts, used as a positive target cell control) were labeled with [51Cr] and incubated for 6 h with cytotoxic B6 anti-BALB/c spleen cells. As demonstrated in Fig. 6B, Con A blasts were predominantly killed by cytotoxic cells, whereas the cells from the 80% Percoll fraction (LSCs) were more resistant to cytotoxicity than were unseparated limbal cells or cells from a 50% Percoll gradient.

Discussion

Using Percoll gradient centrifugation, we were able to isolate a small fraction of limbal cells that demonstrate markers and characteristics attributed to LSC. This cell fraction expressed the LSC markers ABCG2 and p63, was negative for the cornea-associated markers K12 and connexin 43, exhibited a SP phenotype, and had the growth characteristics of SC (21). In this study, we show that these cells with a LSC phenotype: 1) inhibit the proliferation of activated T and B lymphocytes; 2) suppress cytokine production; 3) express enhanced levels of the genes for the antiapoptotic molecules Mcl-1, XIAP, and survivin and for FasL; and 4) are more resistant to CTL-mediated and staurosporin-induced cell death than other limbal cell populations.

In their physiological role, LSCs are responsible for the production of differentiated cell types that ensure corneal epithelial cell renewal and corneal transparency (24, 25). Damage to LSCs due to a local inflammatory immune reaction in the anterior segment of the eye or by cytotoxic agents could be a threat for the viability and function of LSCs. Therefore, mechanisms protecting LSCs can be anticipated. We have shown in this paper that LSCs possess the ability to inhibit lymphocyte proliferation and the production of proinflammatory cytokines. In this respect, LSCs are even more suppressive than the classical CD4+CD25+Foxp3+ Tregs or MSCs. Pilot experiments showed that the suppression by LSC was mediated by released soluble factors. We observed that the suppression of lymphocyte proliferation was preserved when LSCs were separated from the lymphocytes by a semipermeable membrane and that supernatants from cultures of LSC-inhibited lymphocyte proliferation. Because it has been reported that MSC can suppress lymphocyte activation through the production of TGF-β, IL-10, hepatocyte growth factor, NO, PGE2, or indoleamine-2,3-dioxygenase (7, 8, 26, 27), and reviewed in Refs. 28, 29), we have made preliminary attempts to characterize the candidate inhibitory molecule in our system. We found that neither TGF-β, IL-10, NO, nor PGE2 appears to be the suppressive molecule produced by LSCs. Our results are in accord with the observation of Tse et al. (30), who also used unseparated responder cells and found that MSC production of IL-10, TGF-β, PGE2, or tryptophan depletion was not responsible for the
suppression in MLC. Similarly, Rasmusson et al. (31) observed that human MSCs inhibit the proliferation of PBL induced by mitogens and alloantigens by different mechanisms. Thus, more different mechanisms in dependence on the origin of MSCs and the stimulus for cell activation are involved in the suppression mediated by MSCs and LSCs. We have also shown that the suppression by LSCs does not affect early T lymphocyte activation as demonstrated by the normal expression of the early activation markers CD25 and CD69 in T lymphocytes stimulated in the presence of LSCs. This is in contrast with the observations of Le Blanc et al. (32) and Groh et al. (33) and our unpublished observations that the suppression mediated by MSCs attenuated the expression of early activation cell markers (such as CD25, CD38, CD69) in lymphocytes. These different findings underline the functional and phenotypic differences between tissue-specific LSCs and MSCs.

FIGURE 5. Expression of the genes for FasL and the antiapoptotic molecules Mcl-1, XIAP, and survivin by LSC. Freshly isolated limbal tissue was trypsin dissociated, and the cells were separated on a Percoll gradient. The expression of genes for FasL (A), Mcl-1 (B), XIAP (C), and survivin (D) was determined in unseparated limbal cells (Uns.) and in two Percoll gradient fractions, 50% (50) and 80% (LSC), by real-time PCR. The expression of each gene was normalized to the expression of GAPDH. Each bar represents the mean ± SD from three determinations.

Our experiments also showed that adult tissue specific LSCs not only modulate lymphocyte proliferation and cytokine production but are also themselves more resistant to CTL-mediated cytotoxicity by alloreactive lymphocytes than unseparated limbal cells or cells from other non-LSC limbal cell fractions. The enhanced resistance of LSC to cytotoxicity may be another mechanism contributing to their survival in the body. It has already been shown that human embryonic stem cells (ESCs) (34, 35), rat ESC-like cells (36), or MSCs (20) are more resistant to cell-mediated lysis and immune rejection than adult cells. The enhanced resistance of LSCs to cell-mediated lysis might be due to the presence of the FasL molecule on LSCs or to the resistance of SCs to TRAIL receptor-mediated apoptosis (36, 37). Because it has been demonstrated that FasL can protect rat ESCs from rejection in an allogeneic host (38), we measured the level of FasL mRNA in the LSC fraction and in other limbal cells. The highest level of FasL mRNA was found in the cell fraction containing LSCs. Nevertheless, we observed that limbal tissue or LSCs, if grafted into immunocompetent allogeneic recipients, are rejected. This sensitivity to rejection reaction in vivo may be associated with the fact that LSCs growing outside their niche gradually lose the SC markers and characteristics and relatively rapidly differentiate into corneal epithelial cells (A. Lencova, K. Pokorna, A. Zajicova, M. Krulova, M. Filipec, V. Holan, manuscript in preparation).

There are more molecules with antiapoptotic properties that could be expressed in LSCs and could contribute to their resistance to cytotoxicity. Using real-time PCR, we measured the levels of mRNA for Bcl-2, Mcl-1, XIAP, Bcl-XL, and survivin, and we found a strong upregulation of the genes for Mcl-1, XIAP, and survivin in LSCs in comparison with unseparated limbal cells or cells of other limbal cell fractions. We also showed that LSCs were more resistant to cell death induced by alloreactive CTL or by the apoptosis-inducing compound staurosporin than other limbal cell populations. Previous studies with ESC also showed enhanced levels of various antiapoptotic molecules in these SCs (39, 40). The enhanced levels of antiapoptotic molecules in ESCs and LSCs may contribute to the resistance of SCs to apoptosis and to CTL-mediated cell death. It has been shown that ESCs (34, 35) and MSCs (20) are relatively resistant to rejection in vivo, and this resistance may contribute to their survival under harmful immunological and chemical attacks.

The results thus demonstrate that cells with LSC markers and characteristics suppress proinflammatory immune reactions and are...
themselves more resistant to apoptotic cell death than other adult cell populations. The resistance to cell death may be associated with the enhanced expression of genes for FasL and the anti-apoptotic molecules McI-1, XIAP, and survivin in LSC. Taken together, our results and studies on ESCs and MSCs (19, 34, 35) suggest that the immunomodulatory and self-protecting properties of LSCs are the general properties of SCs and may contribute to their survival in the body.

Disclosures

The authors have no financial conflicts of interest.

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