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Subpopulation Composition and Activation of T Lymphocytes during Coculturing with Mesenchymal Stromal Cells in Medium with Different O_2 Content

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The concentration of O_2 during coculturing practically did not affect the subpopulation composition of T lymphocytes ($CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD3^+/CD16^+/CD56^+$ T cells) under conditions of PHA-induced activation. Coculturing with mesenchymal stromal cells (MSC) led to a significant decrease in the ratio of lymphocytes carrying activation markers ($CD3^+/CD25^+$ and $CD3^+/HLA\text{-}DR^+$) and increase in the number of $CD3^+/CD16^+/CD56^+$ T cells. The percent of activated $HLA\text{-}DR^+$ T cells in a heterotypic culture with MSC at 5% O_2 was much lower than that observed under normal conditions of culturing (20% O_2). Our results suggest that antigen presentation by T lymphocytes due to HLA-DR expression can be reduced in the target tissues at low concentration of O_2, while the interaction between allogeneic MSC probably contributes to more significant inhibition of the immune response.

Key Words: *T lymphocytes; mesenchymal stromal precursor cells from adipose tissue; reduced oxygen content*

Mesenchymal stromal precursor cells (MSC) hold much promise for cell therapy, in particular, due to their immunomodulatory properties [4,8,11], *e.g.* suppression of T lymphocyte activation and proliferation. The interaction of MSC with immunocompetent cells is regulated by microenvironmental factors. One of the most important factors is partial oxygen pressure, which modulates some properties of cells (*e.g.*, MSC) [1].

Here we studied the subpopulation composition and activation of T lymphocytes during coculturing in the standard medium (20% O_2) or under conditions of reduced O_2 concentration (5%). These parameters were also evaluated during coculturing with human lipoaspirate MSC.

MATERIALS AND METHODS

Lymphocytes were routinely obtained from the peripheral blood of healthy donors using a Ficoll-Histopaque density gradient (density 1.077; Sigma). MSC were isolated from the adipose tissue by means of enzymatic treatment [2,13] and cultured under standard conditions (20% O_2, 5% CO_2, and 75% N_2; normoxia) or reduced oxygen concentration (5% O_2, 5% CO_2, and 90% N_2; hypoxia).

Experiments were performed on passage 2-4 MSC. Intact and PHA-stimulated lymphocytes (10 μg/ml) in a concentration of 10^6 cells/ml were maintained in

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RPMI 1640 medium with 5% inactivated FBS. This mixture was put in flasks containing 70-80% monolayer of MSC precultured at 20 and 5% O_2 or flasks without cells. The cells (monocultures of lymphocytes; and heterocultures of lymphocytes and MSC) were cultured at 20 and 5% O_2 for 72 h. Lymphocyte suspensions were taken from all flasks. The subpopulation composition of T lymphocytes and cell activation with monoclonal antibodies (BD) were studied on an Epics XL flow cytofluorometer (Beckman Coulter) according to manufacturer's recommendations. We estimated the ratio of $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD3^+/CD16^+/CD56^+$, $CD3^+/HLA\text{-}DR^+$, and $CD3^+/CD25^+$ cells. Cell viability was evaluated with Annexin_V_Fitc-PI kit (Immunotech).

The results were analyzed by nonparametric Mann–Whitney test.

RESULTS

Lymphocyte viability was studied after culturing at various concentration of O_2. The percentage of viable lymphocytes after 72-h culturing in the absence of PHA was 90-93%. The ratio of cells was reduced to 60% under conditions of activation (Table 1). Our results are consistent with published data on standard parameters of cultured lymphocytes. Cell viability did not depend on the concentration of O_2.

The ratio of $CD3^+/CD4^+$ cells decreased slightly during lymphocyte activation. These changes practically did not depend on culturing conditions (presence or absence of MSC). However, the observed changes were statistically significant during normoxia. The percentage of $CD3^+/CD8^+$ lymphocytes increased significantly upon PHA-induced activation at 20 and 5% O_2 (by 1.5 times). The changes were less pronounced in heterotypic cultures of activated T cells and MSC (1.2-fold increase) and did not differ from those observed during coculturing of nonactivated lymphocytes with MSC. Activation was also accompanied by an increase in the ratio of $CD3^+/CD16^+/CD56^+$ T cells during normoxia and hypoxia. Coculturing with MSC was followed by a greater increase in the number of these cells.

Activation of T cells was estimated from the percentage of lymphocytes expressing the early and late activation markers (CD25 and HLA-DR, respectively; Table 2).

The ratio of $CD25^+$ cells was very low (2%) in the absence of PHA. Coculturing of lymphocytes and MSC was followed by a 2-fold increase in the percentage of these cells. PHA-induced activation was accompanied by a significant increase in the ratio of $CD25^+$ cells. However, the number of these cells was reduced by 2 times during coculturing with MSC. Similar changes were observed at various concentrations of O_2 in the medium. Treatment with PHA at 20 and 5% O_2 was accompanied by a significant increase in the ratio of $HLA\text{-}DR^+$ lymphocytes. These changes were less significant during hypoxia than during normoxia. During coculturing with MSC, the percentage of $HLA\text{-}DR^+$ lymphocytes decreased slightly at 20% O_2 and by 2 times at 5% O_2. Our results indicate that the decrease in the concentration of O_2 in the culture medium has little effect on the ratio between T cell populations. However, activation of these cells in a monoculture or heterotypic culture (coculturing with MSC) was less pronounced at low concentration of O_2.

MSC hold much promise for regenerative medicine. Immune privilege and immunosuppressive activity determine the possibility for allogeneic administration of these cells. These properties of MSC were studied in details [4,8,9,12]. The interaction of MSC with immune cells occurs at high level of Po_2 (administration into systemic circulation) and reduced Po_2 in the target organs. Immunocompetent cells (primarily T lymphocytes) are well adapted to variations in Po_2 in the microenvironment. During the life cycle, these cells migrate from lymphoid tissues (very low concentration of O_2) to the peripheral blood (high con-

TABLE 1. T Lymphocyte Subpopulations at Various Concentrations of O_2 in the Culture Medium ($M\pm m$; n=5)

Ratio (relative to the number of T cells), %	Normoxia, 20% O_2				Hypoxia, 5% O			
	lymphocytes	+PHA	+MSC	+PHA, +MSC	lymphocytes	+PHA	+MSC	+PHA, +MSC
$CD3^+/CD4^+$	51.5±1.5	47.6±0.7*	52.2±0.7	42.5±0.9^x	52.1±2.5	49.9±1.6	52.3±2.0	46.2±2.4
$CD3^+/CD8^+$	27.5±1.2	44.8±4.9*	27.0±1.9	32.2±10.2	26.4±1.3	38.8±3.9*	23.8±0.5	29.6±3.5
$CD3^+/CD16^+/CD56^+$	3.3±0.9	6.8±2.1	3.8±1.7	13.4±1.5^{+x}	3.0±1.5	6.0±1.4	2.0±0.3	14.8±1.7^{+x}

Note. Here and in Table 2: p<0.05: *compared to lymphocytes; $^+$compared to lymphocytes+PHA; ^{x}compared to lymphocytes+MSC; ^{o}compared to normoxia.

TABLE 2. Expression of Markers for T Lymphocyte Activation after Culturing at Various Concentrations of O_2 in the Medium ($M\pm m$; n=5)

Ratio (relative to the number of T cells), %	Normoxia, 20% O_2				Hypoxia, 5% O_2			
	lympho-cytes	+PHA	+MSC	+PHA, +MSC	lympho-cytes	+PHA	+MSC	+PHA, +MSC
$CD3^+/CD25^+$	2.3±0.3	65.4±6.9*	4.0±0.5*	46.0±4.2^{+x}	2.5±0.3	70.2±6.4*	3.8±0.1	46.2±3.7^{+x}
$CD3^+/HLA\text{-}DR^+$	2.1±0.6	15.3±4.3*	2.2±0.8	13.1±1.6^{x}	1.2±0.3	11.5±1.4*^{o}	1.4±0.0	7.5±0.5^{xo}

centration of O_2). Then these cells will continue to move into the target tissues, where Po_2 can vary significantly (up to anoxia) [10]. The immune response mediated by T cells is inhibited during *in vivo* [10] or *in vitro* hypoxia [3,6,7,10]. Our *in vitro* study showed that the ratio between T cell subpopulations remains practically unchanged in response to the decrease in O_2 concentration. It probably results from the ability of T lymphocytes to adapt to Po_2 variations. We found that hypoxia has an inhibitory effect only on one parameter of T cell activation, expression of the histocompatibility antigen HLA-DR. These data suggest that suppression of the immune response during hypoxia is partially related to a decrease in the ratio of T cells, which can present foreign antigens to other immunocompetent cells. The majority of *in vitro* studies for immunosuppressive properties of MSC were performed at a normal concentration of O_2 in the culture medium (20%) [4,9,12]. The MSC-mediated decrease in T cell activation (HLA-DR antigen) under normoxic conditions can be potentiated by MSC that are cultured at the reduced concentration of O_2 (5%).

These data extend the knowledge on the interaction between stromal progenitor cells and immunocompetent cells in the adult organism. Moreover, we showed that hypoxia plays an important role in T cell activation. Our results suggest that the ability of T lymphocytes for antigen presentation due to HLA-DR expression will be significantly reduced in tissues after administration of allogeneic MSC. Expansion of these cells can result in the development of hypoxia. These changes have a favorable effect on tissue reparation and/or engraftment.

RESULTS

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