MICROBIOLOGY AND IMMUNOLOGY

Number of Stromal Precursors in Mouse Bone Marrow and Expression of Cytokine Genes in Primary Cultures of Mouse Bone Marrow Cells during Various Periods after Immunization with *S. typhimurium* Antigens

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Administration of *S. typhimurium* microbial mass to mice was followed by a significant increase (by 3-4 times) in the efficiency of cloning and number of stromal precursors in the femoral bone marrow. These parameters were maximum on days 1-3, but returned to normal by the 8th-15th day after immunization. As differentiated from intact animals, the expression of genes for proinflammatory cytokines IL-1β (day 1 after immunization), IL-6 (days 1-3), TNF-α (days 1, 3, and 6), and IFN-α (days 1-3) was detected in bone marrow cultures from immunized mice. The expression of genes for IFN-γ, IL-18, and IFN-α was decreased on days 1, 3, and 6 after immunization of animals, respectively. Gene expression for the anti-inflammatory cytokine IL-4 was observed on day 6 after immunization. Therefore, this system was not characterized by a decrease in the immune response of stromal cells. The stromal component of hemopoietic and lymphoid organs has the vector of influences in response to bacterial antigens. This vector is directed to the stimulation and progression, but not to the suppression of immune reactions. Our results indicate that resident stromal cells play a role in the immune response of the body.

Key Words: *stromal cells; immune response; cytokine mRNA*

In vitro and *in vivo* administration of stromal cells (SC) suppresses the immune response of lymphocytes to transplant antigens and mitogens [11]. SC of hemopoietic and lymphoid organs provide specific microenvironment for proliferation and differentiation of hemopoietic and lymphoid cells. It can be suggested that these cells play a role in the immune response in the body. Previous studies showed that immunization of animals is accompanied by a sharp increase in the number of stromal precursors (CFU-F) in the lymph nodes (by several times), spleen (by 10 times) [1,2], and bone marrow under conditions of repeated immunization with type 5 streptococcus A antigens [3]. These changes do not impair the immune response in the corresponding organs. Mesenchymal stem cells (MSC) express Toll-like receptors (TLR)

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1, 2, 3, 4, 5, 6, and 9, respond to TLR ligands, and play a role of antigen-presenting cells upon stimulation with IFN-γ [10].

It remains unclear whether resident SC of lymphoid organs can maintain the immune response of the body (as differentiated from exogenous SC).

This work was designed to study the effect of immunization with *S. typhimurium* on the efficiency of cloning (ECF-F) and number of CFU-F in the bone marrow of animals during various periods after immunization. We also evaluated the effect of this treatment on the synthesis of mRNA for anti-inflammatory and proinflammatory cytokines in primary cultures of bone marrow cells from immunized mice (as compared to intact animals).

MATERIALS AND METHODS

Experiments were performed on adult CBA mice weighing 18-20 g. Some animals were immunized by an intraperitoneal injection of *S. typhimurium* microbial mass (400 μg in 0.4 ml physiological saline). The suspension of bone marrow cells from intact and immunized specimens was prepared as described elsewhere [4]. To estimate ECF-F, bone marrow cells ($1\text{-}3\times10^6$ cells) from intact and immunized mice (days 1, 3, 7, and 15 after immunization) were explanted in flasks (bottom area 25 cm^2). On days 10-12, these cultures consisted of discrete colonies of stromal fibroblasts and admixture of macrophages. The cultures were fixed with ethanol and stained with azure-eosin (Giemsa technique). We calculated the colonies with at least 50 fibroblasts. ECF-F was estimated from the number of grown colonies (*i.e.*, count of colonies after explantation of 10^6 cells). Bone marrow cells from intact and immunized mice were explanted ($5\times10^6\text{-}1\times10^7$ cells per flask) to study the expression of cytokine genes in test cultures. After 8-10 days, these cultures included the sublayer of fibroblasts (confluent growth of stromal precursors). They also consisted of macrophages and small amounts of hemopoietic and lymphoid cells. The cultures were grown in an incubator at 5% CO_2. The culture medium was removed by the end of incubation. The expression of cytokine mRNA was measured in these cultures.

Reverse transcription-PCR assay (RT-PCR) was used to evaluate activity of mRNA of the following ten cytokines in cell cultures: IFN (IFN-α and IFN-γ), IL (IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-18), and TNF-α. RNA was isolated by extraction with guanidine thiocyanate, phenol, and chloroform [5]. RT and PCR-amplification were performed as described previously [7]. Experiments were conducted with primer pairs for IFN-α [7], IL-6, IL-1β, IL-2, IL-4, IL-10, TNF-α, IFN-γ [12], IL-18 [6], and IL-12 [8]. β-Actin was used as a positive control [9]. The results of PCR were recorded electrophoretically in 2.5% agarose gel after staining with ethidium bromide. Nucleotide sequences were identified with an electrophoresis marker G 1758 (Promega).

RESULTS

Administration of *S. typhimurium* microbial mass to the body was followed by a significant increase (by 3-4 times) in ECF-F and number of bone marrow stromal precursors in the femur of mice (Table 1). These parameters were maximum on days 1-3, but returned to normal by the 8th-15th day after immunization. As differentiated from intact animals, the expression of genes for proinflammatory cytokines IL-1β (day 1 after immunization), IL-6 (days 1-3), TNF-α (days 1, 3, and 6), and IFN-α (days 1-3) was detected in bone marrow cultures from immunized mice. The expression of genes for IFN-γ, IL-18, and IFN-α decreased on days 1, 3, and 6 after immunization of animals, respectively. Gene expression for the anti-inflammatory cytokine IL-4 was observed on day 6 after immunization (Table 2). Therefore, this system was not characterized by a decrease in the immune response of stromal cells. The stromal component of hemopoietic and lymphoid organs has a vector of response to bacterial antigens directed to stimulation and progression, but not to suppression of immune reactions. Our results indicate that resident stromal cells play a role in the immune response of the body.

TABLE 1. ECF-F in Cultures of Bone Marrow Cells from Intact and Immunized Mice (*M*±*m*)

Time after immunization, days	Number of nucleated cells per organ, $\times10^7$	ECF-F, $\times10^{-5}$	Number of CFU-F per organ
Intact	1.3±0.2	1.6±0.2	215±19
1	1.2±0.2	7.5±1.2	900±128
3	1.1±0.2	6.5±0.7	715±73
8	1.1±0.1	3.0±0.3	330±65

TABLE 2. Effect of Immunization with *S. typhimurium* on the Synthesis of Cytokine mRNA in Primary Cultures of the Bone Marrow from Intact and Immunized Mice

Time after immunization, days	IFN		IL							TNF-α
	α	γ	1β	2	4	6	10	12	18	
Intact	+	+	-	+	-	-	+	+	+	-
1	+	- ↓	+ ↑	+	-	+ ↑	+	+	+	+ ↑
3	+	+ ↑	- ↓	+	-	+	+	+	- ↓	+
6	- ↓	+	-	+	+ ↑	- ↓	+	+	+ ↑	+

Note. «+», presence of cytokine mRNA; «-», absence of cytokine mRNA. ↑, appearance of mRNA; ↓, disappearance of mRNA.

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