# Reaction of Bcl-2-Positive Splenic Cells to Glucocorticoids

**E. M. Luzicova and O. A. Efimova**

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Study of the glucocorticoid effects on the counts of Bcl-2-positive cells in various zones of the spleen showed that dexamethasone and prednisolone stimulated migration of apoptosis-resistant cells to the spleen, but their effects on cell distribution in various morphofunctional zones of the spleen were different. The population of Bcl-2-positive cells is divided into morphotypes, differing by location and reaction to glucocorticoids.

**Key Words:** *spleen; Bcl-2-positive cells; glucocorticoids*

Study of the molecular mechanisms of apoptosis is a pressing problem of immunology today. The complex Bcl-2 protein family is considered to be the main regulator of apoptosis, extremely important for tissue development and homeostasis and protecting them from pathogenesis. The *bcl-2* gene product Bcl-2 protein is the priority suppressor of apoptosis. It is located on the cytoplasmatic surface of the mitochondrial outer membrane, on the endoplasmatic reticulum and nuclear membrane and can register damage to these structures and modify their behavior, presumably by modulating the transport of minor protein molecules [8]. Due to its double function (ionic channel and adaptor protein), Bcl-2 is an important suppressor of apoptosis [7]. Under optimal growth conditions, Bcl-2 provides cell transition to a silent status and delays the onset of the mitotic cycle. The expression of antiapoptotic proteins in the peripheral immune organs is conjugated with protection from apoptosis during several days, needed for the development of immune response [3]. High counts of Bcl-2-positive cells were found among dying cells [11].

It is known that immature T- and B-lymphocyte precursors, double-negative thymocytes, mature long-living T- and B-lymphocytes are Bcl-2-positive [1]. Splenic Bcl-2-positive cells are little studied. We failed to find data on the morphotypes of Bcl-2-positive cells and their distribution in the splenic parenchyma.

Glucocorticoid hormones are classical apoptosis inductors. It was found that glucocorticoid hormones induced overall death of the splenic white pulp lymphocytes. Lymphocytes of the deep red pulp zone are less sensitive to these hormones [4]. A characteristic feature of glucocorticoids is immunosuppressive activity. In contrast to cytostatics, the immunosuppressive effects of glucocorticoids are not due to their mitostatic action, but result from suppression of the immune reaction at different stages: inhibition of bone marrow stem cell and B-lymphocyte migration, suppression of T- and B-lymphocyte activity, and inhibition of cytokine release (IL-1, IL-2, γ-IFN) from leukocytes and macrophages. In addition, glucocorticoids reduce the formation and increase the degradation of the complement system components, block the immunoglobulin Fc-receptors, suppress leukocyte and macrophage functions. Glucocorticoid hormones cause changes in the counts of macrophages and mast cells in various morphofunctional zones of the spleen [2]. Published data on glucocorticoid effects on Bcl-2 expression are contradictory. According to some reports, Bcl-2 protects

Laboratory of Medical Biology Department, Chuvash State University, Cheboksary, Russia. ***Address for correspondence:*** nema76@mail.ru. E. M. Luzicova

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the cells from dexamethasone-induced apoptosis [5], according to other authors, Bcl-2 initiates apoptosis induced by glucocorticoids in lymphoid cells *in vivo* [6].

We studied the expression of Bcl-2 protein in various morphofunctional zones of the spleen in health and during glucocorticoid treatment.

## MATERIALS AND METHODS

The spleens of 2-month-old male mice ($n$=45) were examined. The animals were divided into 2 groups receiving different treatment: 1) isotonic saline ($n$=15); 2) a single dose (0.3 mg) of dexamethasone ($n$=15); and 3) a single dose (0.3 mg) of prednisolone ($n$=15). The spleens were collected under deep chloroform narcosis 1 h after injection. All experimental manipulations were carried out in accordance with "Regulations for Studies on Experimental Animals". After fixation in 10% neutral formalin the material was dehydrated in ascending ethanols and embedded in paraffin. Paraffin sections (5 μ) were sliced on an MPS-2 paraffin rotator microtome. Immunohistochemical detection of Bcl-2 was carried out by the standard biotin-streptavidin-peroxidase method using LSAB-2 kit (Labeled Streptavidin Biotin System Peroxidase) with anti-Bcl-2 rabbit polyclonal antibodies. The specificity of immune staining was evaluated using a negative control for each test (treatment by control antibodies instead of the first antibodies, giving no immune staining). At the final stage the sections were poststained by Toluidine Blue after Niessle.

Quantitative distribution of stained cells was evaluated by counting them in 10 visual fields of Micmed-5 microscope at ×1000 and subsequent estimation of the mean arithmetic. The resultant values were statistically processed using DVM486DX-2 software (Microsoft Office). The statistical significance was evaluated using Student's $t$ test.

## RESULTS

Normally Bcl-2-positive cells in the spleen are present in the deep and peripheral layers of the red pulp (PRP), in the splenic lymphoid nodule mantle zone (MZ), rarely in the periarterial lymph muffs (PALM). A frame of Bcl-2-positive cells (5-7 rows) formed outside the marginal sinus in the perifollicular zone of the red pulp (PZRP). The mean number of Bcl-2-positive cells per visual field is 25.3 in intact and 28.5 cells in control animals. Prednisolone elevates this value to 46.4, dexamethasone to 48.5 cells per visual field. Dexamethasone causes an increase in the counts of Bcl-2-positive cells in PALM (2.1 times) and in the red pulp (2.25 times in PRP and 1.6 times in PZRP). The increase in the number of Bcl-2-positive cells in PALM is a result of increased migration of apoptosis-resistant cells to the spleen. The increase in the number of studied cells in the red pulp is also primarily a result of migration; however solitary Bcl-2-expressing cells, dividing by mitosis, are seen. Quantitation of lymphocytes showed that dexamethasone promoted an increase in lymphocyte counts in the red and white pulps of the spleen (1.6 times; Fig. 1). As expression of Bcl-2 in the cells protected the adjacent Bcl-2-negative cells [11], presumably, our data can explain the resistance of the splenic red and white pulp lymphocytes to a single exogenic dose of dexamethasone. Prednisolone significantly increased the number of cells expressing Bcl-2 in PALM and splenic lymphoid nodule MZ (5.5 and 5.7 times, respectively). Despite stimulation of migration of Bcl-2-positive cells to the spleen, the number of lymphocytes reduces in the red and white pulp in response to prednisolone 1.2 and 1.3 times, respectively, presumably because of reduction (3.9 times) in the content of Bcl-2-positive cells in PZRP. The differences in the effects of dexamethasone and prednisolone on Bcl-2 expression can be due to difference in the drug efficiencies (the former is 7-fold more active) and, presumably, binding to different glucocorticoid receptors, differing by molecular weight, affinity for the hormone, and other physicochemical characteristics. After penetration through the membrane inside the cell glucocorticoids bind to receptors, which leads to activation of heterocomplexes, including also Hsp-90 and Hsp-70 heat shock proteins, immunofilin, *etc.* The oligomeric protein complex is dissociated: Hsp-90 and Hsp-70 and immunofilin se-

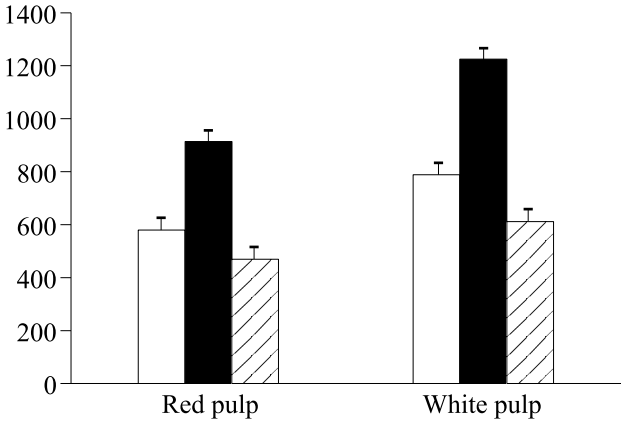


**Fig. 1.** Changes in the counts of splenic lymphocytes under the effects of glucocorticoids. The mean counts per visual field at ×1000 are shown. Light bars: control; dark bars: dexamethasone; cross-hatched bars: prednisolone.

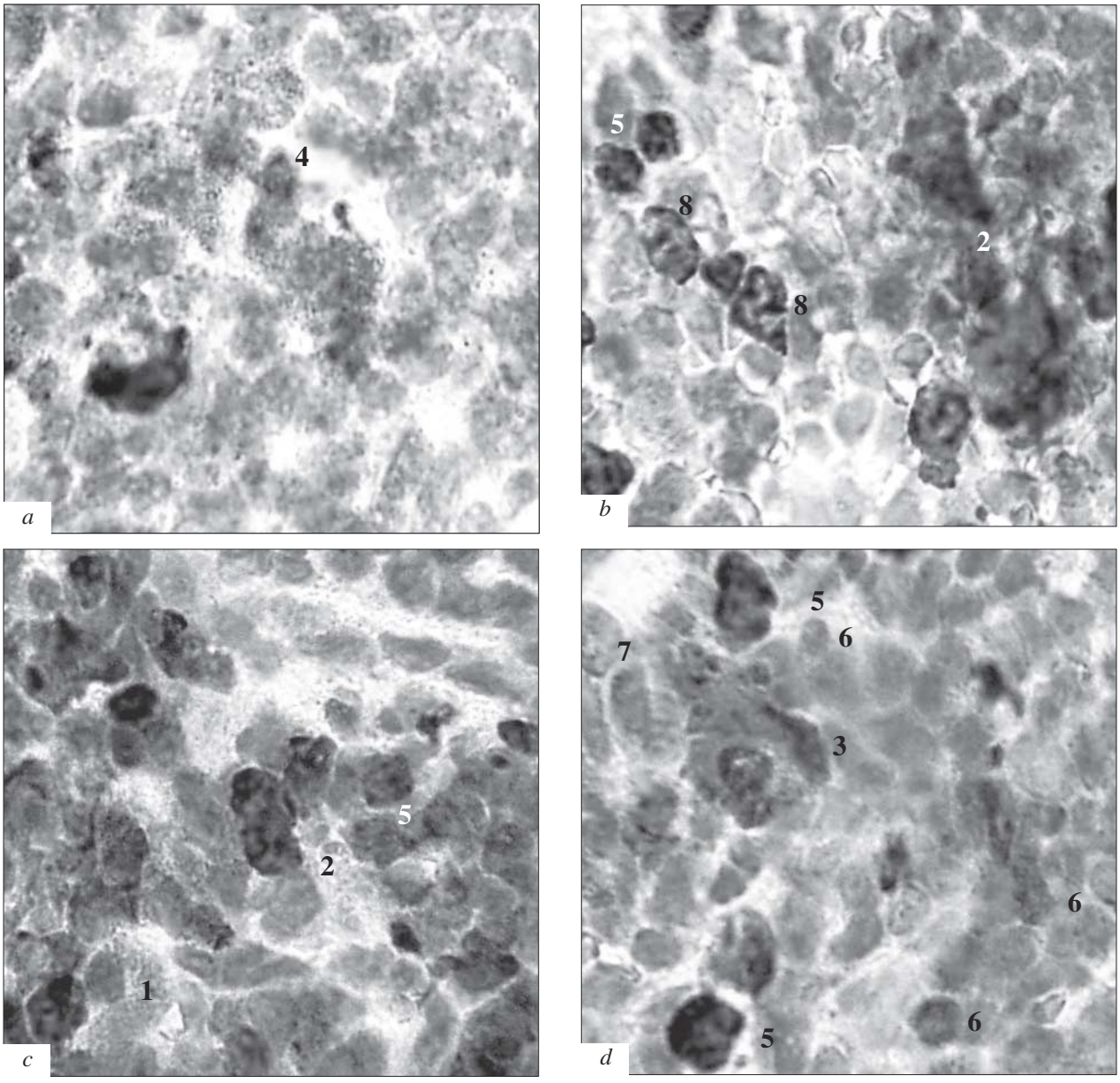


**Fig. 2.** Immune staining by anti-Bcl-2 rabbit polyclonal antibodies with post-staining by Toluidine Blue after Niessle, ×1000; Bcl-2-positive cells in the splenic red pulp of control animals. *a*) morphotype 4; *b*) morphotypes 2, 5, 8; *c*) morphotypes 1, 2, 5; *d*) morphotypes 3, 5, 6, 7.

parate. As a result, the receptor protein (a monomer when in the complex) acquires the capacity to become a dimer. The resultant glucocorticoid-receptor complexes are transported into the nucleus, where they react with DNA sites located in the promotor fragment of steroid-reacting gene and regulate (activate or inhibit) the transcription of certain genes. This leads to stimulation or suppression of mRNA formation and modification of synthesis of some regulatory proteins and enzymes. Hence, dexamethasone and prednisolone enhance the expression of Bcl-2 in the spleen, but their effects on the counts of lymphocytes in the red and white pulps are different, presumably because of different genomic effects determining the cell effects.

Based on the findings of visual and morphometrical studies, we distinguished the following types of Bcl-2-positive cells:

1) medium-sized cells ($S_m$=250.0±2.7) with a nucleus at the periphery and cytoplasm containing many medium-sized bright granules;
2) large ($S_m$=332.00±0.65) or medium-sized ($S_m$=273.0±1.5) axonal cells with a nucleus in the center and cytoplasm with many large bright granules;

**TABLE 1.** Morphotypes of Bcl-2-Positive Splenic Cells Normally and after Glucocorticoid Treatment

| Morphotype | Percentage of Bcl-2-positive cells | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | control | | | | dexamethasone | | | | prednisolone | | | |
| | MZ | PALM | PRP | PZRP | MZ | PALM | PRP | PZRP | MZ | PALM | PRP | PZRP |
| 1 | 0 | 10.0±1.3 | 11.0±2.3 | 4.0±0.1 | 10±2* | 24.0±1.8* | 18.0±1.8* | 15.0±0.4* | 22±1* | 19.0±1.8* | 10±2 | 13.0±1.5* |
| 2 | 22.0±1.5 | 21.0±2.5 | 16.0±1.1 | 12.0±0.4 | 0 | 1.0±0.1** | 10.0±2.3* | 12.0±2.2 | 0 | 16.0±2.2* | 15±3 | 10±1* |
| 3 | 26±1 | 26.0±1.8 | 18.0±0.5 | 14.0±1.5 | 57.0±3.2* | 31.0±3.9 | 22.0±2.5 | 27±3* | 52.0±6.2** | 31.0±1.6* | 30.0±2.8** | 29.0±3.6** |
| 4 | 0 | 9.0±0.1 | 12.0±0.2 | 8.0±2.5 | 0 | 0** | 7.0±0.3* | 0* | 0 | 0** | 6.0±1.9* | 0* |
| 5 | 22.0±1.7 | 7.0±0.6 | 11±1 | 9±1 | 3.0±0.6* | 9.0±0.3* | 14.0±1.5* | 17.0±1.5* | 4.0±0.7* | 8±2 | 14.0±1.3* | 13.0±1.5* |
| 6 | 30±2 | 27.0±1.2 | 14.0±1.9 | 46.0±4.9 | 30.0±2.5 | 19±2* | 14.0±2.9 | 15.0±1.8* | 22.0±1.5* | 15.0±2.5* | 11.0±2.4 | 21±2* |
| 7 | 0 | 0 | 9.0±0.5 | 4.0±1.6 | 0 | 5.0±0.2** | 8.0±0.9 | 6.0±0.6 | 0 | 11±2** | 7.0±0.5* | 6.0±0.2* |
| 8 | 0 | 0 | 9.0±1.3 | 3.0±0.1 | 0 | 11±1** | 7.0±1.5 | 8.0±0.8* | 0 | 0 | 7.0±0.8 | 9.0±0.6* |

**Note.** *$p<0.05$, **$p<0.01$ *vs.* control.

3) polygonal cells, large ($S_m$=315.0±4.2) or medium-sized ($S_m$=248.0±3.8) with a nucleus in the center and cytoplasm with many pale small granules;
4) large polygonal cells ($S_m$=312.0±3.2) with few medium-sized bright granules in the cytoplasm;
5) small ($S_m$=70.0±4.7) round cells with poorly discernible nucleus and cytoplasm containing many medium-sized bright granules;
6) small cells with few small granules in the cytoplasm ($S_m$=28.0±2.3);
7) medium-sized ($S_m$=160.0±2.7) round cells with a large bean-shaped nucleus and a narrow rim of the cytoplasm with small pale granules; and
8) medium-sized ($S_m$=186.0±2.6) cells of irregular shape with segmented nucleus and cytoplasm with medium-sized bright granules.

Types 2, 3, and 4 cells resemble macrophageal cells by morphological signs (Fig. 2). Type 5 cells are presumably small, medium, and large lymphocytes; type 7 cells can be referred to monocytes, and type 8 cells are presumably granulocytes. We failed to identify types 1 and 6 cells. All these cell types are present in the splenic red pulp. No Bcl-2-positive cells of types 4, 7, and 8 were detected in the lymphoid nodule MZ normally or after treatment of any kind (Table 1). Type 6 cells are dexamethasone-resistant and type 7 cells prednisolone-resistant in this zone. In the PALM zone dexamethasone had virtually no effect on types 3 and 5 cells, while types 3, 5, and 8 cells were in fact resistant to prednisolone. Types 6 and 7 cells were not sensitive to dexamethasone in PRP and types 1 and 2 were not sensitive to prednisolone in this zone. In the PZRP dexamethasone did not modify the counts of type 2 cells; no prednisolone-resistant cells were detected in this zone. Hence, dexamethasone modified the percentage of Bcl-2-positive cells of types 1, 4, and 8, while prednisolone modified the counts of types 3, 4, and 6 cells in all zones.

Hence, dexamethasone and prednisolone stimulate migration of apoptosis-resistant cells to the spleen, their effects on cell distribution in the morphofunctional zones of the spleen being different. The population of Bcl-2-positive cells is heterogeneous and represented by not only lymphocytes, but also by macrophages, presumably granulocytes and monocytes. The Bcl-2-positive cell morphotypes differ by location and quantitative reaction to glucocorticoids.

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