POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, SI 2, pp. 45–49

EFFECTS OF LYSOZYME DIMER ON THE CELLULAR AND HUMORAL RESPONSE IN HYDROCORTISONE-TREATED MICE

Bożena Obmińska-Mrukowicz, Marianna Szczypka

Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Agricultural University, Wrocław, Poland

Key words: lysozyme dimer, hydrocortisone-induced immunosuppression, cellular and humoral response

The studies were carried out on non-immunized or SRBC-immunized Balb/c mice (8 weeks of age) exposed to immunosuppression by a single *i.p.* dose (125 mg/kg) of hydrocortisone. The mice were immunized *i.p.* with 0.2 mL of 10% sheep red blood cells (SRBC) suspension 24 h after hydrocortisone administration. Lysozyme dimer at a dose of 20 μg/kg was administered to non-immunized mice once or four times at 24-h intervals prior to hydrocortisone injection or once 2 h prior to SRBC or four times at 24-h intervals after antigen stimulation in SRBC-immunized mice. It has been found that hydrocortisone injection to non-immunized mice decreased the percentage of immature $CD4^+CD8^+$ thymic cells with a corresponding increase in the percentage of mature $CD4^+$ and $CD8^+$ thymic cells. In addition, the percentage of $CD3^+$ and $CD4^+$ mesenteric lymph node cells was reduced. The administration of hydrocortisone (125 mg/kg) to SRBC-immunized mice decreased the number of splenocytes producing haemolytic antibodies (PFC) and discontinued the production of total and 2-mercaptoethanol resistant anti-SRBC haemagglutinins. Lysozyme dimer (20 μg/kg) administered four times to non-immunized mice prior to hydrocortisone injection reduced its suppressive action on the percentage of immature $CD4^+CD8^+$ thymic cells. Moreover, pre-treatment with lysozyme dimer potentiated the increasing effect of hydrocortisone on the percentage of mature $CD4^+$ and $CD8^+$ thymocytes. The strong effect was noted after a single injection of the drug. Lysozyme dimer administered prior to hydrocortisone, irrespective of the number of doses applied, increased the percentage of $CD3^+$ and $CD4^+$ splenocytes, and counteracted the suppressive action of hydrocortisone on the percentage of $CD3^+$ lymph node cells with a corresponding increase in the percentage of $CD8^+$ cells. Pre-treatment with lysozyme dimer did not change the suppressive action of hydrocortisone on the percentage of $CD4^+$ mesenteric lymph node cells. Lysozyme dimer administered to SRBC-immunized mice after their exposure to pharmacological immunosuppression leads to partial restoration of PFC and their capability of producing anti-SRBC haemagglutinins. The restorative action of lysozyme dimer did not depend on the number of doses applied.

INTRODUCTION

Glucocorticoids are well known suppressants of almost all cell-mediated immune events. Enhanced production of endogenous glucocorticoids or administration of exogenous steroids has a direct thymolytic impact, and are able to induce apoptosis of corticosterone-sensitive, double-positive cortical thymocytes and inhibit the endocrine activity of thymic epithelial cells, thereby attenuating the differentiation and maturation of intra- and extra-thymic T lymphocytes [Cohen, 1992; Green *et al.*, 1992; McConkey *et al.*, 1992]. Moreover, it has been found that reduced activities of T lymphocytes due to steroid treatment are connected with the suppressive effect of these agents on the synthesis and release of interleukin-1 (IL-1), interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [Homo-Delarche & Durant, 1994].

Lysozyme dimer is a highly purified semi-synthetic substance, obtained by the chemical polymerization of natural N-acethylmuramylohydrolase of hen's egg white. It is the active ingredient of Lydium-KLP (Nika Health Products, Switzerland), an immunomodulating drug used in veterinary practice. Preclinical trials *in vitro* have revealed that lysozyme dimer, depending on its concentration, is able to enhance the production of IFN-α and modulate the synthesis and release of IL-2, interleukin-6 (IL-6) and TNF-α through the culture of human lymphocytes stimulated by concanavalin A (Con-A) [Klein & Kiczka, 1994]. Moreover, the results obtained *in vivo* show that lysozyme dimer is also able to modulate the synthesis and release of IL-1 by murine peritoneal macrophages stimulated *in vitro* with lipopolysaccharide from *E. coli* (LPS). The modulating effect of the drug depends on the dose as well as the consecutive doses administered [Obmińska-Mrukowicz *et al.*, 2002]. Earlier studies by the same authors conducted on SRBC-immunized mice have confirmed the potentiating effect of lysozyme dimer on the primary and secondary humoral response in mice. The studies proved that there is a relationship between the immunological effect induced by lysozyme dimer and the dose of the drug as well as the time of administration in relation to priming and challenge [Obmińska-Domoradzka *et al.*, 1997a, 1998]. Moreover, the protective action of lysozyme dimer on the humoral response of SRBC-immunized mice was found in mice which has been immunosuppressed with a high dose of cyclophosphamide (200 mg/kg). The protective action of

Author's address for correspondence: Bożena Obmińska-Mrukowicz, Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Agricultural University, Norwida 31, 50-375 Wrocław, Poland; e-mail: m.mrukowicz@triangulum.pl

lysozyme dimer is also time- and dose-dependent [Obmińska-Domoradzka *et al.*, 1997 b].

The purpose of the present study was to determine the dose-dependent ability of lysozyme dimer to restore the cellular and humoral response impaired by a high dose of hydrocortisone (125 mg/kg).

MATERIALS AND METHODS

Animals. The studies were conducted on male and female Balb/c mice, each weighing 18–20 g (8–10 weeks of age). The experimental animals were obtained from a breeding laboratory at the Medical University, Wrocław, Poland. The part of mice were immunized *i.p.* with 0.2 mL of 10% sheep red blood cells (SRBC) suspension (4×10^8 cells per mouse). The sheep blood was collected into Alsever's solution in sterile manner and kept at 4°C for at least 3 days. The SRBC suspension was prepared *ex tempore* in phosphate buffered saline (PBS).

The animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs and treatment. Lysozyme dimer (Lydium-KLP, Nika Health Products, Switzerland, series 659602) at a dose of 20 μg/kg was administered intraperitoneally once or four times at 24-h intervals in non-immunized mice. However, in SRBC-immunized mice lysozyme dimer (series 644596) was administered intraperitoneally once 2 h prior to antigen stimulation or four times at 24-h intervals, *i.e.* 2 h before and then 24, 48 and 72 h after SRBC immunization.

Pharmacological immunosuppression was induced by a single intraperitoneal hydrocortisone injection (*Hydrocortisonum hemisuccinatum*, Jelfa, series 40797) administered at a dose of 125 mg/kg after the last lysozyme dimer exposure in non-immunized or 24 h prior to antigen stimulation in SRBC-immunized mice.

The trials in the control groups were conducted parallely. The mice received phosphate buffered saline (PBS) instead of lysozyme dimer. The volume of each dose of lysozyme dimer, hydrocortisone (HC) or PBS was 0.2 mL per animal. Each experimental group consisted of eight mice.

Assay of thymocyte, splenocyte and lymphocyte of mesenteric lymph node subpopulations. CD subsets of thymocytes, splenocytes and lymphocyte of mesenteric lymph node were determined twice: 1 and 5 days after hydrocortisone injection. The mice were anaesthetized with halothane 24 h after the last lysozyme dimer injection. The thymuses, spleens and mesenteric lymph nodes were removed and placed in disposable Petri dishes containing sterile, ice-cold phosphate buffered saline solution (PBS). The suspended cells were released from the lymphatic organs by being passed through a nylon mesh and then centrifuged on a layer of Ficoll 400 (Pharmacia, Fine Chemicals AB, Uppsala, Sweden)/Uropolinum 75% (diatrizoate sodium and meglumine diatrizoate; Polpharma S.A., Starogard Gdański, Poland) at a 1:3 ratio, density 1.071. After centrifugation at 4°C, the cells were collected from the interphase and washed twice with PBS supplemented with 1% bovine serum albumin (BSA, Sigma) at 4°C. After the second wash the cells were suspended in PBS with 1% BSA at 1×10^7 cells/mL. The viability of each cell suspension was determined by trypan blue dye exclusion, and was found at a level of 90–94%. The cells were resuspended in 100 μL PBS solution with 1% BSA. The thymocytes, splenocytes and lymphocytes of mesenteric lymph nodes were stained with FITC-labelled antibody to mouse $CD4^+$ clone: YTS 177.9 (lot. 7169-03S, BioSource) and PE-labelled antibody to mouse $CD8^+$ clone: KT 15 (lot. 14353-02S, BioSource) at a dilution recommended by the producer. The splenocytes and lymphocytes of mesenteric lymph nodes were also stained with FITC-labelled antibody to mouse $CD3^+$ clone: KT3 (lot. 19887-01S, BioSource) and PE-labelled antibody to mouse $CD19^+$ clone: 6D5 (lot. 16249-02S, BioSource) at a dilution recommended by producer.

The cells were incubated at 4°C for 30 min and washed 3 times with ice-cold PBS buffer. Fluorescence was analysed using a FACS Calibur (Becton Dickinson) flow cytometer. Thymocyte, splenocyte and lymphocyte marker distribution was analysed using the Cell Quest program.

Determination of plaque forming cells (PFC). The number of PFC was determined on days 4 and 7 after SRBC injection. The mice were killed by cervical dislocation. The spleens were removed and placed in the Hank's saline. The spleens were teased apart with forceps by gently tearing the capsule and releasing the cells. The suspended cells were centrifuged on a layer of Ficoll 400/Uropolinum 75% (density 1.071). After centrifugation at 4°C, the splenocytes were collected from the interface and washed twice in the Hank's saline. After the second wash the splenocytes obtained were suspended in the Hank's saline, so that the number of cells in 1 mL was 1×10^6. Afterwards, viability of the isolated splenocytes was evaluated in an *ex tempore* prepared mixture consisting of 0.2% trypan blue and 4.25% NaCl. The viability of the splenocytes suspensions was 96–100%. The splenocytes producing haemolytic anti-SRBC antibodies (PFC) were determined by the method of local haemolysis in agar gel as described by Mishell and Dutton [1967].

Determination of anti-SRBC antibodies in the serum. Anti-SRBC haemagglutinin titre was determined on days 4, 7, 10 and 14 after SRBC immunization. The blood samples were taken from retro-ocular arteria of halothan anaesthetized mice. The sera were obtained by blood centrifugation. They were next inactivated at 56°C for 30 min. The total and 2-mercaptoethanol resistant serum agglutination titres were defined by active haemagglutination test as described by Adler [1965], carried out on microplates. The titre of 2-mercaptoethanol resistant antibody is roughly equivalent to that due to IgG in the serum, and so the greater titre obtained without 2-mercaptoethanol is due to the IgM. It was checked that serum non-immunized mice did not contain spontaneous anti-SRBC antibodies.

Statistical analysis. The data obtained in the study were subjected to a statistical analysis using a Student t-test. The differences were considered significant at $p<0.05$.

RESULTS

The effect of lysozyme dimer on thymocyte, splenocyte and lymphocyte of mesenteric lymph node subpopulations in hydrocortisone-suppressed mice

As shown in Table 1, a single injection of hydrocortisone (125 mg/kg) decreased the percentage of immature $CD4^+CD8^+$ thymic cells (double positive thymocytes). The suppressive action of hydrocortisone was maintained for 5 days. In contrast, only 1 day after hydrocortisone injection a temporary increase in the percentage of mature $CD4^+$ and $CD8^+$ thymocytes (single positive cells) was observed. At the same time, some changes in the percentage of mesenteric lymph node lymphocyte were found. On days 1 and 5 the percentage of $CD3^+$ and $CD19^+$ was observed. The administration of a single dose of hydrocortisone did not affect the percentage of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ splenocytes.

The administration of a single dose of lysozyme dimer (20 μg/kg) prior to hydrocortisone did not change the suppressive effect of this steroid on the percentage of immature double-positive $CD4^+CD8^+$ thymocytes as early as 1 day following the exposure to glucocorticoid. On day 5 the suppressive effect of hydrocortisone on the percentage of $CD4^+CD8^+$ thymic cells was increased by a single injection of lysozyme dimer. In contrast, lysozyme dimer (20 μg/kg) injected four times prior to exposure to hydrocortisone, decreased its suppressive effect on the percentage of immature $CD4^+CD8^+$ thymic cells. Pre-treatment with lysozyme dimer intensify the increasing effect of hydrocortisone on the percentage of mature $CD4^+$ thymocytes only one day after steroid injection. A strong effect was noted after one injection of lysozyme dimer.

Pre-treatment with lysozyme dimer prior to hydrocortisone injection, irrespective of the number of the dose applied increased the percentage of $CD3^+$ and $CD4^+$ splenocytes. On the other hand, administration of lysozyme dimer one or four times before hydrocortisone injection did not change its suppressive effect on the percentage of $CD3^+$ and $CD4^+$ mesenteric lymph node cells as early as 1 day following the exposure to steroid. Lysozyme dimer reduced the suppressive action of the steroid on the percentage of $CD3^+$ mesenteric lymph node cells with corresponding increase in the percentage of $CD8^+$ cells, but did not change the suppressive action of hydrocortisone on the percentage of $CD4^+$ mesenteric lymph node cells.

Pre-treatment with lysozyme dimer (20 μg/kg), irrespective of the number of doses applied did not change the percentage of $CD19^+$ splenocytes and mesenteric lymph node cells in hydrocortisone-suppressed mice.

The effect of lysozyme dimer on the humoral response to SRBC in hydrocortisone-immunosuppressed mice

As reported in Figure 1, a single dose of hydrocortisone (125 mg/kg) injected 24 h prior to SRBC decreased the number of splenocytes producing haemolytic antibodies (PFC) and discontinued the production of total and 2-mercaptoethanol resistant anti-SRBC haemagglutinins. The suppressive action of hydrocortisone was observed for 14 days. Lysozyme dimer (20 μg/kg) administered to mice 24 h after their exposure to pharmacological immunosup-

TABLE 1. Percentage of thymocyte, splenocyte and mesenteric lymph node cell subpopulations in hydrocortisone-suppressed mice treated with lysozyme dimer (20 μg/kg) once or four times at 24 h intervals. The mean value (n=8) and standard deviation.

Index	Day	Control	HC	LyD 1x + HC	LyD 4x + HC
Thymus					
% $CD4^+CD8^+$	1	80.3±4.9	57.6±15.6○	49.9±8.5○●	61.2±9.1○
	5	82.3±4.7	68.3±10.5○	50.7±10.2○●	79.6±3.0●
% $CD4^-CD8^-$	1	4.4±3.8	10.7±2.6○	10.1±5.1○	9.3±3.9○
	5	4.4±3.5	8.6±2.8	12.3±7.3○●	5.6±2.2
%$CD4^+CD8^-$	1	11.7±1.8	17.6±1.6○	31.9±6.8○●	22.1±7.6○●
	5	10.6±1.8	13.0±2.3	14.9±3.3○	11.4±1.4
%$CD4^-CD8^+$	1	3.5±0.9	5.5±1.6	7.8±1.5○	7.0±1.7○
	5	3.9±2.9	5.9±2.9	15.0±6.4○●	6.2±1.3
Spleen					
%$CD3^+$	1	18.4±3.0	18.2±4.6	26.4±6.1○●	24.6±2.9○●
	5	18.9±3.1	17.3±5.9	19.9±2.4	22.1±4.4○●
%$CD4^+$	1	15.2±1.9	15.5±3.3	22.1±4.4○●	18.6±3.7○●
	5	16.6±4.8	16.1±4.5	18.4±2.5	16.5±2.2
%$CD8^+$	1	5.8±0.9	5.9±1.0	7.1±2.0○●	6.1±0.7
	5	5.1±1.0	6.2±0.7	7.3±0.9○	8.3±2.6○●
%$CD19^+$	1	74.2±1.7	71.2±4.0	68.9±7.5	68.6±2.4
	5	73.4±1.7	70.5±8.2	70.6±1.8	68.3±4.5
Mesenteric lymph node					
%$CD3^+$	1	46.2±6.8	37.1±7.2○	35.7±9.2○	37.3±4.4○
	5	47.1±4.8	40.6±2.3○	45.6±6.8●	44.4±6.0●
%$CD4^+$	1	39.9±6.1	32.4±4.3○	30.2±9.0○	31.5±2.8○
	5	40.2±5.1	31.6±8.1○	28.0±4.7○	29.0±2.6○
%$CD8^+$	1	8.4±2.7	8.0±2.6	7.9±1.7	9.6±1.7
	5	8.1±1.9	10.2±3.7	17.3±7.4○●	14.1±4.8○●
%$CD19^+$	1	51.7±6.8	50.2±6.2	59.8±4.8○●	56.5±4.8○●
	5	50.9±6.1	47.4±7.5	46.9±11.9	54.9±7.5

HC – hydrocortisone, LyD – lysozyme dimer; ○ – $p<0.05$ as compared to the control group; ● – $p<0.05$ as compared to hydrocortisone group

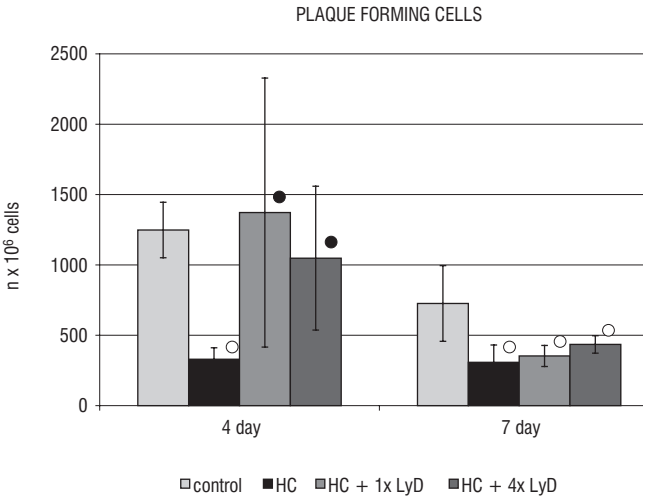

FIGURE 1. Number of plaque forming cells (PFC) in SRBC-immunized mice treated with lysozyme dimer (20 μg/kg) once or four times at 24-h intervals after hydrocortisone (125 mg/kg) injection. LyD – lysozyme dimer, HC – hydrocortisone. The mean value (n=8) and standard deviation. ○ – significantly different from control group, $p<0.05$, • – significantly different from hydrocortisone group, $p<0.05$

pression leads to total restoration of PFC on day 4, but did not change the suppressive effect of hydrocortisone on the number of PFC on day 7.

Lysozyme dimer injected after hydrocortisone partially counteracted the suppressive action of steroid in the production of anti-SRBC haemagglutinins (total and 2-mercaptoethanol resistant). The restorative action of lysozyme dimer did not depend on the number of the consecutive doses applied (Figures 2 and 3).

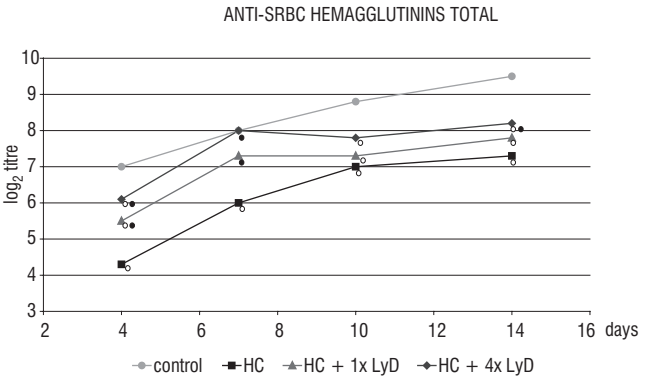

FIGURE 2. Serum total titre in SRBC-immunized mice treated with lysozyme dimer (20 μg/kg) once or four times at 24-h intervals after hydrocortisone (125 mg/kg) injection. LyD – lysozyme dimer, HC – hydrocortisone. The mean value (n=8) and standard deviation. ○ – significantly different from control group, $p<0.05$, • – significantly different from hydrocortisone group, $p<0.05$

DISCUSSION

The present trials conducted on non-immunized and SRBC-immunized mice have confirmed the restorative effect o lysozyme dimer administered to mice prior to or after hydrocortisone immunosuppression. Lysozyme dimer treatment prior to a high hydrocortisone dose (125 mg/kg)

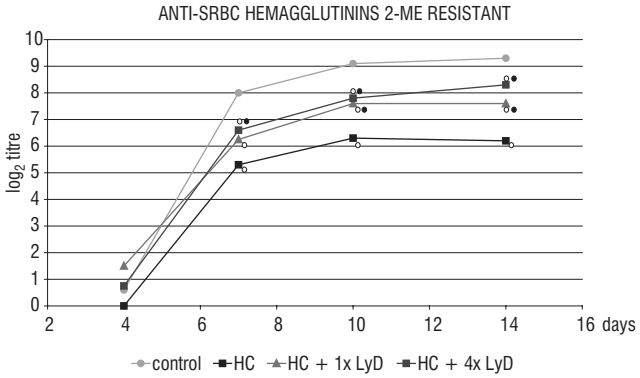

FIGURE 3. Serum 2-mercaptoethanol-resistant titre in SRBC-immunized mice treated with lysozyme dimer (20 μg/kg) once or four times at 24-h intervals after hydrocortisone (125 mg/kg) injection. LyD – lysozyme dimer, HC – hydrocortisone. The mean value (n=8) and standard deviation. ○ – significantly different from control group, $p<0.05$, • – significantly different from hydrocortisone group, $p<0.05$

partially counteracted the immunosuppressive action of steroid. The protective action of the drug is expressed by accelerated regeneration of the percentage of immature $CD4^+CD8^+$ thymocytes, $CD3^+$ and $CD8^+$ splenocytes and mesenteric lymph node cells. The strongest immunocorrecting effect was noted after four injections of lysozyme dimer at a dose of 20 μg/kg. It is very likely that immunomodulating action of lysozyme dimer is due to its direct inducing impact on the differentiation and maturation of intra- and extrathymic T lymphocytes. Earlier studies by the same authors indicate that lysozyme dimer administered to mice increased the percentage of mature $CD4^+$ thymocytes and $CD3^+$ splenocytes and lymph node cells with corresponding augmented in the percentage of $CD4^+$ and $CD8^+$ cells. The stimulating effect of lysozyme dimer depends on the dose as well as the consecutive doses applied [Obmińska-Mrukowicz *et al.*, 2002]. On the other hand, the protective action of lysozyme dimer can be attributed to the activation of T lymphocytes and monocytes through the cytokine cascade (IL-1, IL-2, IL-6, TNF-α and INF-α) enhanced by this drug [Klein & Kiczka, 1994; Siwicki *et al.*, 1997].

The present paper also shows that the humoral response of SRBC-immunized mice reduced by high hydrocortisone dose (125 mg/kg) can be partially restored by lysozyme dimer. However, the same authors have found, that lysozyme dimer has a capability to intensify the primary and secondary humoral response of SRBC-immunized mice. This effect is expressed as the increase of the number of murine splenocytes producing haemolytic anti-SRBC antibodies (PFC) and the rise of the level of total and 2-mercaptoethanol resistant anti-SRBC antibodies. In those studies, a relationship between the adjuvant action of lysozyme dimer on the primary and secondary response to SRBC and the time- and dose-schedules with respect to antigen stimulation was observed [Obmińska-Domoradzka *et al.*, 1994, 1997, 1998]. The mechanism of the adjuvant action of lysozyme dimer is still unknown, but it seems quite likely that it is associated with the activation of monocytes capable of producing IL-6 and T lymphocytes able to produce IL-2. At present, it is assumed that IL-6 stimulates the differentiation of antigen-stimulated B lymphocytes to the cells producing antibodies of the IgM and IgG class

[Muraguchi *et al.*, 1988]. In addition, IL-6 affects T lymphocytes, inducing the synthesis of the receptor for IL-2 and stimulating the production of this cytokin [German *et al.*, 1987]. It is well known that IL-2 induces the production of IL-4 and IL-5 by T lymphocytes changing the synthetized immunoglobulin class from IgM into IgG, both present in the primary and secondary humoral response [Mahanty & Nutman, 1993; Smith, 1993]. Moreover, the immunocorrective effect of lysozyme dimer on the primary humoral response was observed in old aged mice [Obmińska-Domoradzka & Szczypka, 2001]. This study indicates that the humoral response to SRBC was weaker in old aged mice than in young. In addition, the protective action of lysozyme dimer on the humoral response to SRBC was also found in SRBC-immunized mice which had been immunosuppressed with a high cyclophosphamide dose (200 mg/kg). The protective effect of the drug is time- and dose-dependent, but to the largest extent it depends on the time of its administration in relation to pharmacological immunosuppression [Obmińska-Domoradzka *et al.*, 1997b]. The immunocorrecting action of lysozyme dimer can be associated with the stimulation of synthesis and release of cytokine such as IL-1, IL-2 and IL-6 as well as induction of markers differentiating T lymphocytes. The studies of Schwarze [1977] indicate that reduced antibody production due to cyclophosphamide treatment can be restored after the activation of T-helper-inducer lymphocytes, but the activation of B lymphocytes does not cause such effect.

CONCLUSIONS

In conclusion, it can be stated that lysozyme dimer administered to hydrocortisone-suppressed mice is only partially capable of restoring the reactivity of some immunological responses impaired by glucocorticoids.

REFERENCES

1. Adler F.L., Studies in mouse antibodies II. Mercaptoethanol-sensitive 7S antibodies in mous antisera to protein antigens. J. Immunol., 1965, 95, 26–47.
2. Cohen J.J., Glucocorticoid-induced apoptosis in the thymus. Semin. Immunol., 1992, 4, 363–369.
3. German R.D., Jacobs K.A., Clark S.C., Raulet D.H., B-cell stimulatoru factor 2 (2 interferon) function as a second signal for interleukin-2 production by mature murine T cells. Proc. Natl. Acad. Sci. USA, 1987, 84, 7629–7633.
4. Green D.R., Bissonnette R.P., Glynn J.M., Shi Y., Activation-induced apoptosis in lymphoid system. Sem. Immunol., 1992, 4, 379–388.
5. Homo-Delarche F., Durant S., Hormones, neurotransmitters and neuropeptides as modulators of lymphocyte function. 1994, *in*: Immunopharmacology of Lymphocytes (ed. Rola-Pleszczyński M.). Academic Press, London, pp. 169–240.
6. Klein P., Kiczka W., Information on the results of preclinical tests of KLP-602 (dimerized lysozyme). Życie Weterynaryjne 1994, 69,142–145.
7. Mahanty S., Nutman T., The biology of interleukin-5 and its receptor. Cancer Invest., 1993, 11, 624–634.
8. McConkey D.J., Jondal M., Orrenius S., Cellular signaling in thymocyte apoptosis. Sem. Immunol., 1992, 4, 371–377.
9. Mishell R.J., Dutton R.N., Immunization of dissociated spleen cells cultures from normal mice. J. Exp. Med., 1967, 126, 423–442.
10. Muraguchi A., Hirano B., Tang B., Matsuda T., Horil Y., Nakajima K., Kishimoto T., The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J. Exp. Med., 1988, 167, 332–344.
11. Obmińska-Domoradzka B., Szczypka M., Effects of lysozyme dimer on humoral response to sheep erythrocytes in old aged mice. Comparison with calf thymus extract. Pol. J. Vet. Sci., 2001, 4, 3, 140.
12. Obmińska-Domoradzka B., Świtała M., Dębowy J., Kiczka W., Garbuliński T., Effects of lysozyme dimer on humoral response to sheep erythrocytes in non-treated and cyclophosphamide-immunosuppressed mice. J. Vet. Med., 1997b, B 44, 591–598.
13. Obmińska-Domoradzka B., Świtała M., Dębowy J., Modulation of secondary antibody response in SRBC-immunized mice by lysozyme dimer. Immonopharm. Immunotoxic., 1997a, 19, 489–498.
14. Obmińska-Domoradzka B., Świtała M., Kiczka W., Dębowy J., Garbuliński T., Modulating effect of Lydium-KLP on humoral response of SRBC-immunized mice. Pol. J. Pharm., 1994, 46, 358–359.
15. Obmińska-Domoradzka B., Świtała M., Kostka M., Grabowski T., Dębowy J., Effects of lysozyme dimer on secondary humoral response of SRBC-immunized mice. Pol. J. Vet. Sci., 1998, 1, 23–27.
16. Obmińska-Mrukowicz B., Szczypka M., Gawęda B., Modulation of murine macrophages and T lymphocytes by lysozyme dimer. Pol. J. Vet. Sci., 2002, 5, 237–241.
17. Schwarze G., Drug-induced immunological unresponsiveness: selective inhibition of T-cell helper function by cyclophosphamide in mice pretreated with phytohaemagglutinin. Clin. Exp. Immunol., 1977, 27, 178–182.
18. Siwicki A.K., Pejsak Z., Studnicka M., Klein P., Mokrzycka A., Rymuszka A., Bownik A., Badania porównawcze nad wpływem dimeru lizozymu (KLP-602, Lydium-KLP) na komórkowe i humoralne mechanizmy obronne oraz poziom cytokin u prosiąt. Mat. II Krajowego Sympozjum Immunologów Weterynaryjnych. Świnoujście, 8–10 May 1997, p. 175 (in Polish).
19. Smith K.A., Lowest dose interleukin-2 immunotherapy. Blood, 1993, 81, 1414–1423.