EXPERIMENTAL STUDIES

CHEMICAL CHAPERONE TUDCA SELECTIVELY INHIBITS PRODUCTION OF ALLERGEN-SPECIFIC IgE IN A LOW-DOSE MODEL OF ALLERGY

D.B. Chudakov*, O.A. Shustova, O.D. Kotsareva, A.A. Generalov, M.S. Streltsova, Yu.D. Vavilova, G.V. Fattakhova

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry,
16/10 Miklukho-Maklaya str., Moscow, 117997 Russia; *e-mail: boris-chudakov@yandex.ru

The cellular response to endoplasmic reticulum (ER) stress accompanies plasma cell maturation and is one of triggers and cofactors of the local inflammatory response. Chemical chaperones, low-molecular substances that eliminate pathological ER stress, are proposed as means of treating pathologies associated with ER stress. The aim of this study was to evaluate the effect and mechanisms of influence of chemical chaperones on the humoral response in a low-dose model of allergy. The allergic immune response was induced in BALB/c mice by repeated administration of ovalbumin at a dose of 100 ng for 6 weeks. Some animals were injected with both the antigen and the chemical chaperones, TUDCA (tauroursodeoxycholic acid) or 4-PBA (4-phenylbutyrate). Administration of TUDCA, but not 4-PBA, suppressed production of allergen-specific IgE (a 2.5-fold decrease in titer). None of the chemical chaperones affected the production of specific IgG_1. The effect of TUDCA was associated with suppression of the switch to IgE synthesis in regional lymph nodes. This phenomenon was associated with suppressed expression of genes encoding cytokines involved in type 2 immune response, especially *Il4* and *Il9*, which in turn could be caused by suppression of IL-33 release. In addition, TUDCA significantly suppressed expression of the cytokine APRIL, and to a lesser extent, BAFF. Thus, TUDCA inhibition of the allergy-specific IgE production is due to suppression of the release of IL-33 and a decrease in the production of type 2 immune response cytokines, as well as suppression of the expression of the cytokines APRIL and BAFF.

Key words: chemical chaperone; tauroursodeoxycholate; IgE; interleukin 4; interleukin 33; APRIL; BAFF

DOI: 10.18097/PBMC20247001005

INTRODUCTION

The steady increase in the incidence of diseases caused by synthesis of immunoglobulins E (IgE) to harmless allergens, explains enormous efforts of many research groups to find potential means of etiotropic therapy that suppress the formation of IgE-producing B lymphocytes and plasma cells [1]. One of the most promising groups of targets for new therapies are various signaling pathways and molecular mechanisms activated by endoplasmic reticulum (ER) stress, and the ER stress itself. This is explained by several reasons. The first is that selective activation of a part of the signaling pathways associated with ER stress is an obligatory part of the process of B lymphocyte differentiation into plasma cells [2]. Typically, activation of ER stress occurs when incorrectly or incompletely folded proteins accumulate in the ER of cells. In this case, three main signaling pathways are activated: the signaling pathway associated with IRE1α endonuclease, PERK kinase and the transcription factor ATF6α [3]. However, in B lymphocytes, during their differentiation into plasma cells, mainly IRE1α-XBP1- and ATF6α-dependent molecular mechanisms rather than PERK-eIF2α-ATF4 signaling are activated [2–4]. However, the results of recent studies suggest that the formation of local IgE production may also be associated with the activation of PERK-CHOP-dependent signaling [5].

The second reason, by which ER stress signaling pathways represent an attractive target for the treatment of IgE-dependent pathologies, is that they are directly related to the regulation of local allergic inflammation, for example, in asthma [2]. Indeed, ER stress induces the expression of proinflammatory cytokines, such as IL-1β and TNF-α [6]. Under conditions of ER stress, the differentiation of T helper 0 cells shifts towards T helper 2 cells [7].

Abbreviations used: 4-PBA – 4-phenylbutyric acid (4-phenylbutyric acid); AICDA – activation induced cytidine deaminase; APRIL – A proliferation inducing ligand; ATF6α – activating transcription factor 6 alpha; BAFF – B-cell activating factor of proliferation; BHQ-1 – black hole quencher 1; CHOP – C/EBP homologous protein; Ig – immunoglobulin; IL – interleukin; ILC2 – innate type 2 lymphoid cells; IRE1α – inositol requiring enzyme 1 alpha; ELISA – enzyme-linked immunosorbent assay; ER – endoplasmic reticulum; FAM – fluorescein amidite; HRP – horseradish peroxidase; OVA – ovalbumin; PERK – protein kinase R (PKR)-like endoplasmic reticulum kinase; PCR – polymerase chain reaction; PBS – phosphate buffered saline; TACI – transmembrane activator and CAML interactor; TNF-α – tumor necrosis factor alpha; TSLP – thymic stromal lymphopoietin; TUDCA – tauroursodeoxycholic acid; XBP1 – X-box binding protein 1.

Some of the most effective inhibitors of ER stress are the so-called “chemical chaperones”. These are low-molecular substances that prevent unfolding of proteins and formation of their aggregates. There are two main types of such substances: osmolytes (such as glycerol, methylresorcinols, taurine, and trimethylamino-N-oxide) and hydrophobic chaperones (such as 4-phenylbutyrate and taurourosodeoxycholic acid) [8, 9].

The aim of this study was to evaluate the effect of administration of the chemical chaperones tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyrate (4-PBA) on the synthesis of allergen-specific antibodies and to evaluate the potential mechanisms of such effect by using TUDCA as an example.

MATERIAS AND METHODS

Animals

Female BALB/c mice (aged 6–7 weeks) were purchased from the Scientific Center for Biomedical Technologies (Andreevka, Russia). Animals, kept at a 12-h light-dark cycle and housed in cages with filings as bedding, had free access to water and food.

Immunization and Sample Collection

Animals were immunized as in our previous work [10], with the only difference that the immunization period was 6 weeks instead of 4. This was done in order to evaluate the effect of chemical chaperones administered with the antigen over a longer period of time. A total of 34 mice were used in the experiment. Animals were injected with OVA (100 ng/mouse) 3 times a week in the first two weeks and 2 times a week in each of the following weeks. Animals treated with the antigen in the absence of chemical chaperones served as a positive control (n=8). Intact animals (n=8), as well as animals immunized with saline solution (n=6), were used as controls. Some animal along with antigen were administered with 170 mg/kg TUDCA or 70 mg/kg 4-PBA (n=6 in each of these two groups).

At the end of immunization, mice were subjected to blood sampling from the infraorbital sinus to assess specific serum antibody titers and cytokine concentrations, as well as samples of lung tissue and regional lymph node cells were taken to measure gene expression, as described in [11].

Enzyme-Linked Immunosorbent Assay

Analysis of the production of specific IgE and IgG_1 was carried out as described in [11]. To detect specific IgE and IgG_1, we used mouse IgE conjugated with horseradish peroxidase (HRP) (clone 23G3, Abcam, UK) and mouse IgG_1 conjugated with biotin (clone RMG1-1, BioLegend, USA). In the latter case, a conjugate of HRP with streptavidin (BioLegend) was used as a secondary conjugate.

IL-33 and TSLP concentrations were analyzed as follows. A solution of unlabeled commercial antibodies to IL-33 (clone Poly5165) or TSLP (clone 28F12) in PBS (both at 1:1000 dilution) was applied to the wells of 96-well plates and incubated for 18 h at 4°C. After washing, serum samples were applied in blocking buffer (1% BSA in PBS) (at 1:2 dilution) and incubated for 24 h at 4°C. After washing, biotinylated antibodies to IL-33 (clone Poly5165), TSLP (clone 65B12) (both at 1:2000 dilution) were applied. The following antibodies were used: biotin-labeled anti-mouse IL-1α antibodies; biotin-labeled anti-TSLP antibodies. All commercial antibodies for cytokine analysis were obtained from BioLegend. To obtain a calibration curve, standard cytokine samples from the same company were used. The reaction was read as in the case of detection of specific antibodies.

Gene Expression Level Assessment

Real-time PCR was carried out as described previously by means of the BioMaster HS-qPCR kit (BioLabMix, Russia), but using fluorescent probes for product detection [10]. Isolation of mRNA was performed using the ExtractRNA reagent according to the manufacturer's recommendations. Subsequent synthesis of the first strand cDNA was carried out using the OT M-MuLV-RH kit (BioLabMix) according to the manufacturer's instructions. The amplification reaction was carried out 20 μl in the wells of 96-well plates on a CFX Connect device (BioRad) according to the following scheme: primary denaturation for 5 min at 95°C, 50 cycles of denaturation (5 s, 95°C), combined with annealing-amplification (20 s, 64°C). The sequences of the forward (F), reverse primers (R) and fluorescent probes (Z) used (the latter containing 6-FAM at the 5′-end as a fluorophore and BHQ-1 at the 3′-end as a quencher) are shown in the Table 1. Gene expression was simultaneously normalized to the expression of two housekeeping genes, *Gapdh* and *Hprt*.

Statistical Data Processing

All results are shown as means ± SD values. The statistical significance of differences between groups was determined using the ANOVA test with correction for multiple comparisons according to the Sidak method. P-values for this test equivalent to $p<0.05$ corrected for multiple comparisons were considered statistically significant.

RESULTS

In order to determine the effect of chemical chaperones on the production of allergen-specific antibodies, BALB/c mice were immunized for 6 weeks

Table 1. Nucleotide sequences of primers and probes used in this study

Gene		Sequence
Gapdh	F	GGAGAGTGTTTCCTCGTCCC
	R	ACTGTGCCGTTGAATTTGCC
	Z	/6-FAM/-CGCCTGGTCACCAGGGCTGCCATTTGCAGT-/BHQ-1/
Hprt	F	CAGTCCCAGCGTCGTGATTA
	R	TCCAGCAGGTCAGCAAAGAA
	Z	/6-FAM/-TGGGAGGCCATCACATTGTGGCCCTCTGTGTG-/BHQ-1/
germline ε	F	ACCTGGGATCAGACGATGGA
	R	TGCCCTCAGCTAAAAGTGGG
	Z	/6-FAM/-TCCAAGGCTGCTAAGGCCGGGGGTTCCCAC-/BHQ-1/
mIgE	F	CAAGACCCCAGGCCAGC
	R	AGCTCACACTGAGCAGGAAC
	Z	/6-FAM/-TATTGAAGAGGTGGAGGGCGAGGAGCTGGA /BHQ-1/
sIgE	F	CAAGACCCCAGGCCAGC
	R	CTGTCATCCACCTTCCCCAC
	Z	/6-FAM/-CAGCCCCCGAGGTATATGTGTTCCCACCAC-/BHQ-1/
Il4	F	CCATATCCACGGATGCGACA
	R	AAGCACCTTGGAAGCCCTAC
	Z	/6-FAM/-CGGACGCCATGCACGGAGATGGATGTGCCA-/BHQ-1/
Il9	F	TGCTACAGGGAGGGACTGTT
	R	TGGTCGGCTTTTCTGCCTTT
	Z	/6-FAM/-AGCCATGCAACCAGACCATGGCAGGCAACA-/BHQ-1/
Il13	F	GTGTCTCTCCCTCTGACCCT
	R	TCTGGGTCCTGTAGATGGCA
	Z	/6-FAM/-GCCGCTGGCGGGTTCTGTGTAGCCCTGGAT-/BHQ-1/
Il33	F	GGGCTCACTGCAGGAAAGTA
	R	TATTTTGCAAGGCGGGACCA
	Z	/6-FAM/-CCGGCAAAGTTCAGCAGCACCGCAGGCGAA-/BHQ-1/
Tslp	F	GGGCTCACTGCAGGAAAGTA
	R	TATTTTGCAAGGCGGGACCA
	Z	/6-FAM/-CCGGCAAAGTTCAGCAGCACCGCAGGCGAA-/BHQ-1/
Tnfa	F	TCTTCTCAAAATTCGAGTGACAAGC
	R	GATAGCAAATCGGCTGACGGT
	Z	/6-FAM/-GGCTGCCCCGACTACGTGCTCCTCACCCAC-/BHQ-1/
April	F	GGGGCGAAATCTCGGAGAAG
	R	GTTCCATGCGGAGAAAGGCT
	Z	/6-FAM/-GTGGGAGAGGCCTGGAGGCCCAGGGAGACA-/BHQ-1/
Baff	F	TGCCGCCATTCTCAACATGA
	R	GCAAAGATGGGGTCCGTGTA
	Z	/6-FAM/-TGCAGCTGATTGCAGACAGCGACACGCCGA-/BHQ-1/

with a low (100 ng) dose of ovalbumin antigen in the withers area. Analysis of antibody production in the sera of immune animals has shown that TUDCA significantly reduces the production of allergen-specific IgE antibodies (Fig. 1A), but not IgG_1 antibodies (Fig. 1B). There was a significant inhibition of the production of allergen-specific IgE, characterized by a 2.5-fold decrease in titers. At the same time, administration of 4-PBA had an insignificant effect on the production of allergen-specific antibodies (Fig. 1). In this regard, further work was focused on studying mechanisms of the TUDCA effect.

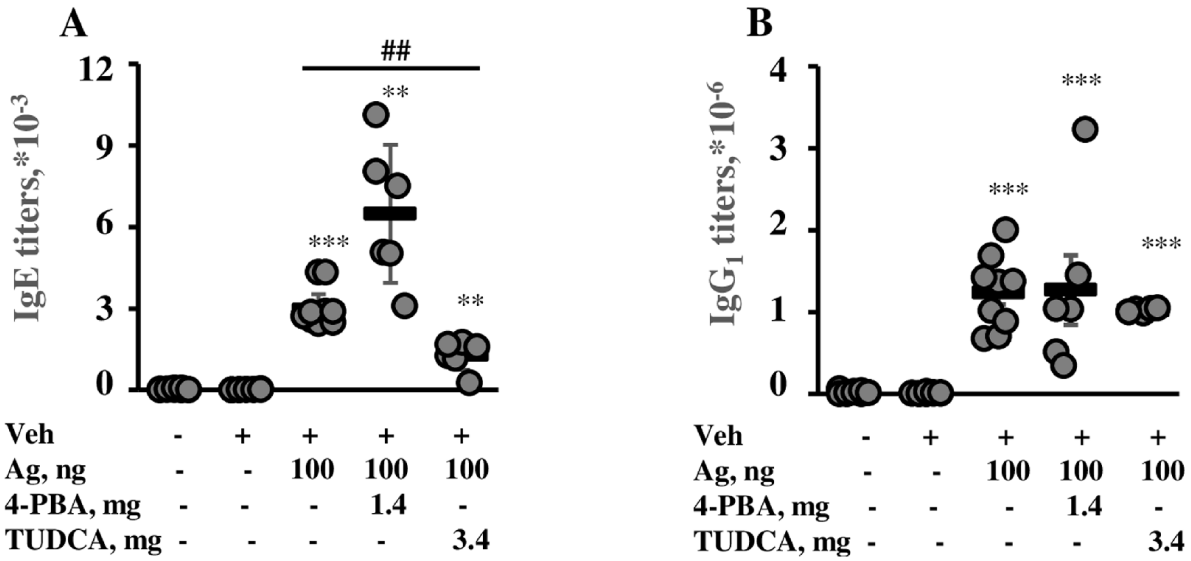

Figure 1. The chemical chaperone TUDCA, but not 4-PBA, selectively inhibits the production of IgE but not IgG_1. BALB/c mice were immunized for 6 weeks with saline solution (Veh), OVA antigen without chemical chaperones (Ag) and with additional introduction of chemical chaperones 4-PBA (4-phenylbutyrate) or TUDCA (tauroursodeoxycholic acid) in the indicated doses to the area withers. Intact animals were used as an additional control. The content of specific IgE (**A**) and IgG_1 (**B**) antibodies was measured in blood serum. */** – $p<0.05/0.01$ significance of the difference in the particular group in comparison with the group of intact animals; #/## – $p<0.05/0.01$ significance of the difference between groups of animals immunized with the antigen with and without chemical chaperone administration.

For this purpose, tissue samples from the immunization site (subcutaneous adipose tissue of the withers region) and regional lymph nodes were taken from immune mice after sacrifice. mRNA was isolated from tissue samples and the expression of various genes and transcripts was analyzed using real-time PCR. According to the data obtained, at later stages, the antigen induced the expression of "germline" transcripts, the markers for the initiation of the process of isotype switching [12], in the cells of regional lymph nodes (Fig. 2A-D). These data differ from those we obtained previously [10], since in this study the duration of immunization was longer (6 weeks instead of 4). TUDCA suppressed the expression of these transcripts in regional lymph nodes (Fig. 2A-D). In addition, in the regional lymph nodes, but not in the tissue of the withers, the expression of the secretory isoform of IgE was expectedly increased under the influence of the antigen, and this expression was suppressed by TUDCA (Fig. 2G,H). In contrast, the inhibitory effect of TUDCA on the membrane isoform of IgE was significant in the withers tissue (Fig. 2E), but not in the lymph nodes (Fig. 2E). The antigen administration did not have a significant effect on the expression of the membrane isoform of IgE, probably due to the fact that the IgE-producers formed under the influence of the antigen quickly differentiated into IgE-producing plasmablasts and plasma cells. However, TUDCA significantly suppressed the basal expression of membrane IgE in the withers tissue (Fig. 2E,F).

Next, we have measured the expression of the genes encoding cytokines involved in type 2 immune response, *Il4*, *Il9*, and *Il13*, as well as the expression of the genes encoding the pro-allergic "tissue" cytokines *Il33* and *Tslp*, which are of great importance in the induction of type 2 immune response [13]. Upon immunization with allergens, disruption of cell integrity, and cellular stress, IL-33 and TSLP are released into the extracellular space [14]. We also measured the concentrations of IL-33 and TSLP in the blood sera of immune mice. The data obtained indicate that the antigen induced of expression of *Il4* in the regional lymph nodes and *Il13* in the adipose tissue of the withers (Fig. 3A,B,E,F). TUDCA increased *Il4* expression in tissue of the withers region, but inhibited the induction of its expression in regional lymph nodes, a site where isotype switching occurred during the later stages (Fig. 3A,B). Under the influence of TUDCA, the induction of *Il13* expression in the tissue of the withers region, but not in the regional lymph nodes, was suppressed as compared with the group of animals immunized with the antigen in the presence of TUDCA and the group of animals immunized with the antigen only (Fig. 4E,F). In addition, the chemical chaperone decreased the basal expression of *Il9* in both regional lymph nodes and withers tissue (Fig. 3C,D). The expression of tissue cytokines was significantly induced by the antigen in the tissue of the withers region, but not in the regional lymph nodes (Fig. 3G-J). TUDCA had no statistically significant effect on *Il33* expression in wither tissue as compared with groups of animals immunized with the antigen in the presence

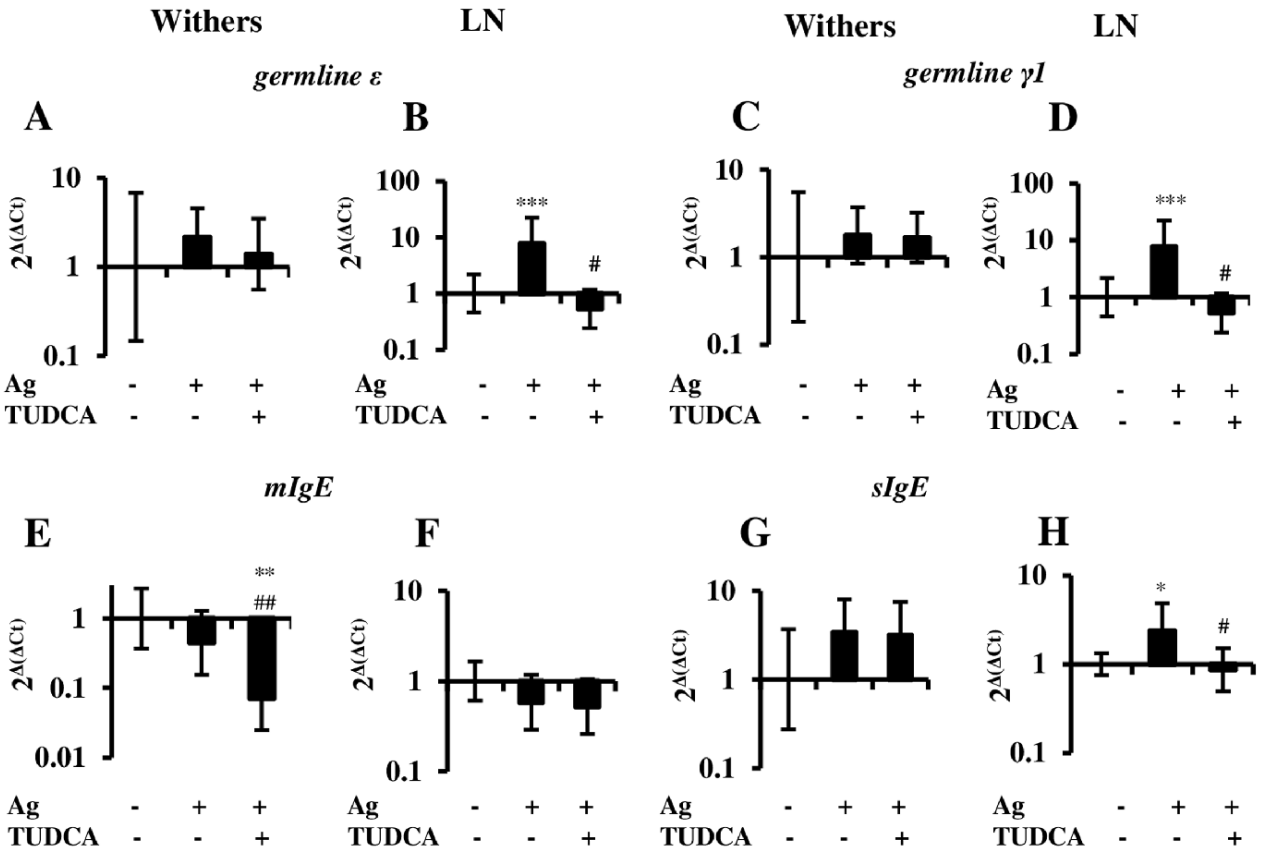

Figure 2. TUDCA inhibited specific IgE production, affecting isotype switching in secondary lymphoid organs but not in the tissue at the site of immunization. BALB/c mice were immunized with saline (control), OVA antigen without TUDCA, or the antigen with TUDCA at the indicated doses for 6 weeks in the withers region. Expression of "germline" ε (**A**, **B**), "germline" γ1 (**C**, **D**), membrane (**E**, **F**) and secretory IgE (**G**, **H**), in the tissue of the immunization site (**A**, **C**, **E**, **G**) and regional lymph nodes (**B**, **D**, **E**, **H**). */** – p<0.05/0.01 significance of the difference in the particular group in comparison with the group of intact animals; #/## – p<0.05/0.01 significance of the difference between groups of animals immunized with the antigen with and without chemical chaperone administration.

and absence of the chemical chaperone. (Fig. 3G). However, a significant difference in *Il33* expression was noted between the group of animals immunized with one antigen and intact animals. No significant difference in *Il33* expression was observed between the group of animals immunized in the presence of TUDCA and the group of intact animals. This indicates that there is a small suppression effect. Indeed, the results of ELISA-based determination of the IL-33 concentration convincingly showed that its release was blocked by TUDCA (Fig. 3K). These results indicate that the effect of TUDCA on the humoral immune response could be due to the blocking of IL-33 release and subsequent suppression of IL-4 and IL-9 expression in regional lymph nodes. Although the PCR results unexpectedly showed the induction of *Tslp* gene expression by TUDCA in regional lymph nodes (Fig. 3L), such induction was not enough for a significant increase of the TSLP concentration in the blood, however, inhibition of TSLP release was also not observed (Fig. 3L).

Cytokines of the TNF family are capable of providing B lymphocytes with a signal that replaces the signal from the CD40 receptor during induction of isotype switching [12], and therefore the next task of the work was to test their expression. The antigen administration induced the expression of the *Tnfa* and *April* genes, but not *Baff*, in the tissue of the withers region, but not in the cells of regional lymph nodes (Fig. 4). Administration of TUDCA significantly blocked the induction of April in the withers tissue and reduced its basal expression in the cells of regional lymph nodes (Fig. 4C,D). Under the influence of TUDCA, there was a decrease in *Baff* expression in regional lymph node cells (Fig. 4F), but not in the subcutaneous fat of the withers region (Fig. 4E). Administration of TUDCA had no significant effect on *Tnfa* expression (Fig. 4A,B). Thus, the effect of TUDCA on the humoral immune response could also be associated with the effect on the expression of cytokines of the TNF family APRIL and BAFF.

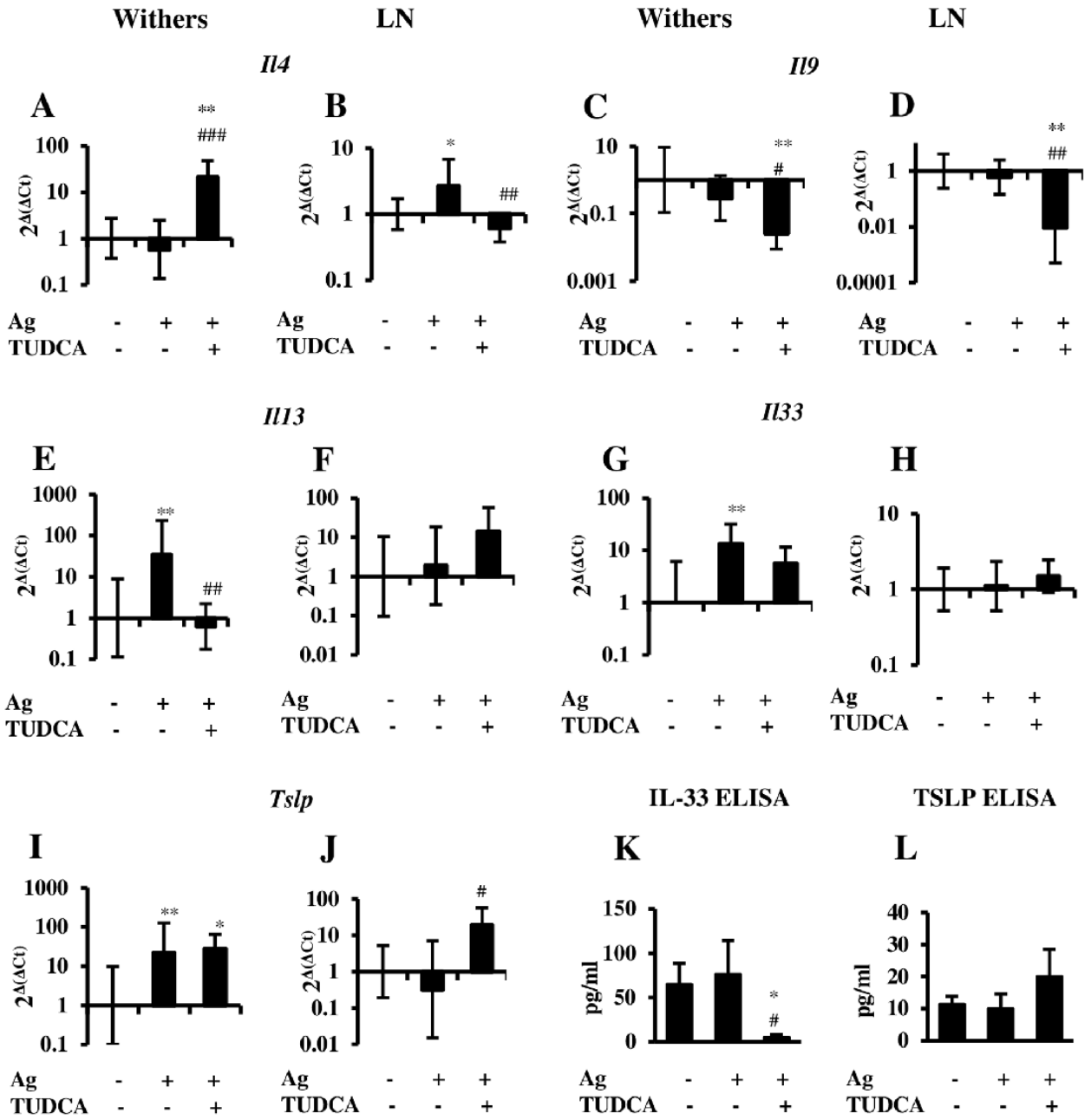

Figure 3. The effect of TUDCA on the humoral immune response is associated with suppression of type 2 cytokine expression in regional lymph nodes and blocking the release of IL-33. BALB/c mice were immunized with saline (control), OVA antigen without TUDCA, or the antigen with TUDCA at the indicated doses for 6 weeks in the withers region. Expression of *Il4* (**A**, **B**), *Il9* (**C**, **D**), *Il13* (**E**, **F**), *Il33* (**G**, **H**), *Tslp* (**I**, **J**) in the subcutaneous fat of the withers region (**A**, **C**, **E**, **G**, **I**) and regional lymph nodes (**B**, **D**, **F**, **H**, **J**). The content of cytokines IL-33 (**K**) and TLSP (**L**) in the blood of immunized animals. */** – p<0.05/0.01 significance of the difference in the specified group in comparison with the group of intact animals; #/## – p<0.05/0.01 significance of the difference between groups of animals immunized with the antigen with and without chemical chaperone administration.

DISCUSSION

The major task of this study was to test the ability of the chemical chaperones TUDCA and 4-PBA to suppress the allergen-specific antibody production. Results of this study indicate that TUDCA, but not 4-PBA, exhibited a pronounced ability to suppress the production of IgE allergen-specific antibodies. However, there was no significant effect on the production of specific IgG_1. In the case of TUDCA, the results obtained are generally consistent with data obtained in studies where TUDCA and other conjugated bile acids suppressed allergic inflammation and the production of specific IgE [15, 16]. Moreover, the effect of TUDCA on the IgE production in the intranasal high-dose model was associated

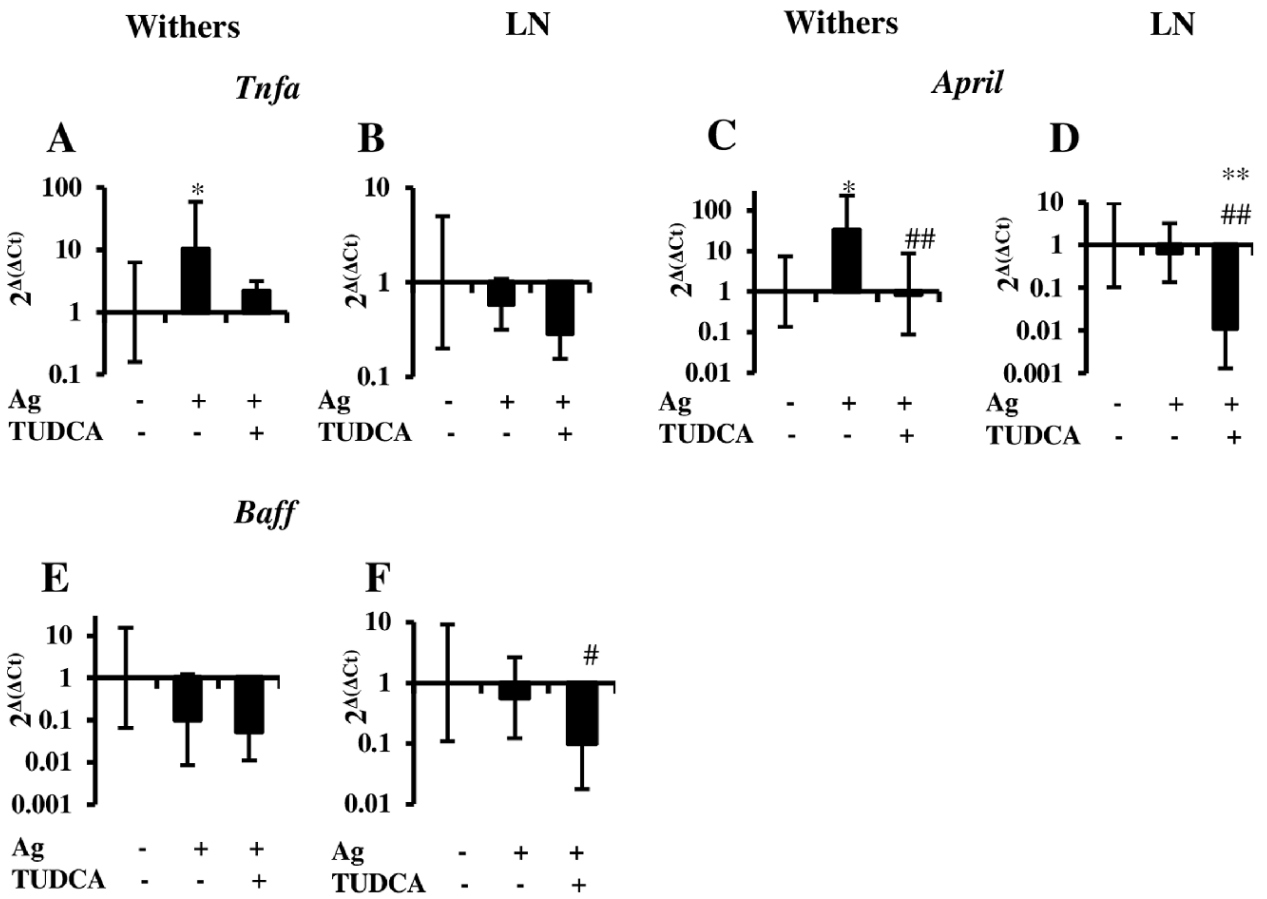

Figure 4. The effect of TUDCA on the humoral immune response is associated with suppression of the expression of TNF family cytokines. BALB/c mice were immunized with saline (control), OVA antigen without TUDCA, or with TUDCA at the indicated doses for 6 weeks in the withers region. Expression of cytokines *Tnfa* (**A**, **B**), *April* (**C**, **D**), *Baff* (**E**, **F**) in the subcutaneous fat of the withers region (**A**, **C**, **E**) and regional lymph nodes (**B**, **D**, **F**). */** – p<0.05/0.01 significance of the difference in the specified group in comparison with the group of intact animals; #/## – p<0.05/0.01 significance of the difference between groups of animals immunized with the antigen with and without chemical chaperone administration.

with the suppression of the activation of ATF6α, an ER stress sensor [15]. The cited works discussed the role of TUDCA in the development of local allergic inflammation in lung tissue, but it was not clearly determined whether the effect of TUDCA was associated with the suppression of the molecular mechanisms of switching B lymphocytes to IgE production in tissues or in regional lymph nodes. The novelty of this work consists not only in the use of a non-adjuvant low-dose model of allergy with a pattern of humoral response more consistent with clinical cases, but also in assessing the effect separately on local and systemic switching. Using real-time PCR, we have shown that, despite the fact that in the early stages the switch of B lymphocytes to IgE synthesis in the model used is carried out locally [11]; at relatively later stages (after 6 weeks of immunization) TUDCA suppresses IgE production, affecting specifically the systemic processes occurring in regional lymph nodes. TUDCA suppressed the expression of "germline" transcripts corresponding to the initiation of the isotype switching process [12] in the cells of regional lymph nodes, but not in the tissue of the immunization site (adipose tissue of the withers). There was also a suppression of the expression of transcripts corresponding to secretory IgE (plasma cells and plasmablasts) in regional lymph nodes. On the other hand, suppression of the expression of the membrane isoform of IgE, characteristic of recently switched B lymphocytes [12], was observed specifically in the withers tissue. This suggests that at relatively later stages of immunization, systemic IgE production plays a more important role. This is the main difference from the results of our previous work, obtained when the biological materials for analysis was taken at earlier stages of immunization, where local switching played a leading role [10].

The second chemical chaperone used (4-PBA) did not have a significant effect on the production of allergen-specific antibodies, probably due to its weaker activity. As was shown in [17], TUDCA, but not 4-PBA, prevented not only the protein

unfolding, but also their aggregation, and under *in vitro* conditions better promoted the survival of cells subjected to ER stress.

Studies [15, 16] proposed one of the possible mechanisms of action of TUDCA, namely, suppression of the association between ATF6α and ER stress mediators Grp78 and, consequently, its proteolysis; this correlated with the inhibition of allergic inflammation and IgE production. It is known that translocation of ATF6 into the Golgi apparatus and its subsequent proteolysis leads to the activation of transcription factors that trigger the expression of the genes *Atf6*, *Erp57*, *Xbp1*, *Chop*, *Grp78*. TUDCA led to suppression of the expression of these genes [16]. XBP1 plays a key role in IL-4-mediated maturation of activated B cells into Ig-producing plasma cells [18].

In regional lymph nodes, TUDCA inhibited the expression of the cytokine IL-4, the main cytokine triggering the switch of B lymphocytes to the synthesis of IgE [12], and IL-9, required for the formation of memory B lymphocytes [19], and also acting as an autocrine factor, supporting the survival of ILC2 cells [20], playing an important role in allergic inflammation. In this case, the expression of IL-4 in the withers, on the contrary, was activated. However, since at the late stages studied, the switch of B-lymphocytes under the influence of antigen to the synthesis of IgE occurred mainly in regional lymph nodes, the above-mentioned phenomenon in the withers tissue was not of great significance. Indeed, according to some data, the most important for switching to IgE synthesis in the late stages of inflammation is IL-4, which is produced directly by T helper cells involved in the formation of immunological synapses with the corresponding B lymphocytes [21].

The effect of TUDCA on the expression of type 2 cytokines is in good agreement with data on activation of signaling pathways associated with ER stress, which required for the differentiation of T-h2 and their production of IL-4, IL-5, and IL-13 [7]. The effect could be explained by the direct negative effect of TUDCA on the activation of ER stress in T helper cells. But the effect can also be explained by an indirect effect on the expression of *Il4*, associated with the regulation of the release of the cytokine IL-33, one of the cytokines with alarmin function, which triggers type 2 immune response [22]. Although TUDCA did not affect the expression of the *Il33* gene either in the tissue of the withers or in the regional lymph nodes, its administration was accompanied by a decrease in the release of IL-33, consistent with the experimental results of the study [15]. Data on the link between ER stress and IL-33 production are quite new.

The switch of B lymphocytes to antibody synthesis requires activation not only of the BCR and not only the presence of "guiding" cytokines, but also signaling from CD40, which, in principle, can replace the activation of TNF family receptors [12]. In the present study, TUDCA did not significantly affect the expression of TNF-α in the tissue of the withers and in the regional lymph nodes as compared with the group of animals immunized with the antigen with the chemical chaperone and the group of animals immunized with one antigen. However, in the group immunized with the antigen and the chemical chaperone, no significant increase in TNF-α expression was observed in comparison with the intact group, in contrast to the group immunized without TUDCA. At the same time, a significant suppression of the expression of APRIL in the tissue of the withers and regional lymph nodes, and BAFF in regional lymph nodes was observed. The greater effect of TUDCA on the expression of BAFF and APRIL may be explained by the difference in the mechanisms of regulation of the expression of these cytokines from the mechanisms of TNF-α expression. These cytokines bind to the TACI receptor and induce a signaling cascade that ultimately activates NF-κB-dependent transcription of the HoxC4 factor, which is a transcriptional regulator of AIDCA [23]. A number of studies have shown that disruption of signal transmission along the APRIL/BAFF-TACI axis prevents the switching of antibody isotypes [24]. In addition, it has been shown that the decrease in the intensity of NF-κB signaling, which can be observed with a decrease in the concentration of APRIL/BAFF, has a particularly critical effect on the switch of B lymphocytes to the IgE isotype, but not IgG_1, since the NF-κB inhibitor in the *ex vivo* system suppressed mainly the first process [25].

Previously, a correlation between the production of these cytokines and the parameters of allergic inflammation and IgE production in patients was shown [26, 27]. The results of this work indirectly confirm the link between the IgE production with the expression of these two cytokines in secondary lymphoid organs and with the expression of APRIL in the tissue of the immunization site.

CONCLUSIONS

Administration of TUDCA, but not 4-PBA, inhibited IgE production in the low-dose model of allergy used. No effect on IgG_1 production was found. This effect of TUDCA is associated with the suppression of systemic, but not local, switching to IgE synthesis, as well as suppression of the IL-33 release, and a decrease in the expression of the *Il4* and *Il9* genes (encoding cytokines involved type 2 immune response) in regional lymph nodes. The effect of TUDCA on the production of allergen-specific antibodies could also be due to a decrease in the expression of the cytokines APRIL and BAFF, which can induce isotype switching in general.

FUNDING

This project was supported by the Russian Science Foundation (project No. 23-25-10044) and by the budget of Moscow.

COMPLIANCE WITH ETHICAL STANDARDS

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (protocol No. 147/2021).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. *Hellman L.* (2007) Regulation of IgE homeostasis, and the identification of potential targets for therapeutic intervention. Biomed. Pharmacother., **61**(1), 34-49. DOI: 10.1016/j.biopha.2006.10.001
2. *Gass J.N., Gunn K.E., Sriburi R., Brewer J.W.* (2004) Stressed-out B cells? Plasma-cell differentiation and the unfolded protein response. Trends Immunol., **25**(11), 17-24. DOI: 10.1016/j.it.2003.11.004
3. *Mesitov M.V., Moskovcev A.A., Kubatiev A.A.* (2013) Molecular logic of signaling pathways during endoplasmic reticulum stress: the UPR (Unfolded Protein Response) system. Pathological physiology and experimental therapy. Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, **57**(4), 97-108.
4. *Ma Y., Shimizu Y., Mann M.J., Hendershot L.M.* (2010) Plasma cell differentiation initiates a limited ER stress response by specifically suppressing the PERK-dependent branch of the unfolded protein response. Cell Stress Chaperones, **15**(3), 281-293. DOI: 10.1007/s12192-009-0142-9
5. *Liu J.-X., Zhen Z., Chen A.-N., Guo C.-L., Shi K.-T., Wang H., Xu K., Yao Y., Wang H., Liao B., Liu Z.* (2021) Endoplasmic reticulum stress promotes local immunoglobulin E production in allergic rhinitis. Laryngoscope Investig. Otolaryngol., **6**(6), 1256-1266.
6. *Kim S., Joe Y., Surh Y.-J., Chung H.T.* (2018) Differential regulation of toll-like receptor-mediated cytokine production by unfolded protein response. Oxid. Med. Cell Longev., **2018**, 9827312. DOI: 10.1155/2018/9827312
7. *Kemp K.L., Lin Z., Zhao F., Gao B., Song J., Zhang K., Fang D.* (2013) The serine-threonine kinase inositol-requiring enzyme 1α (IRE1α) promotes IL-4 production in T helper cells. J. Biol. Chem., **288**(46), 33272-33282. DOI: 10.1074/jbc.M113.493171
8. *Cortez L., Sim V.* (2014) The therapeutic potential of chemical chaperones in protein folding diseases. Prion, **8**(2), 197-202. DOI: 10.4161/pri.28938
9. *Mihajlova V.V., Eronina T.B., Chebotareva N.A., Kurganov B.I.* (2023) The influence of chemical chaperones on protein aggregation processes occurring in various kinetic regimes. Biochemistry (Moscow), **88**(1), 83-96. DOI: 10.31857/S0320972523010062
10. *Chudakov D.B., Kotsareva O.D., Konovalova M.V., Tsaregorotseva D.S., Schevchenko M.A., Sergeev A.A., Fattakhova G.V.* (2022) Early IgE production is linked with extrafollicular B- and T-cell activation in low-dose allergy model. Vaccines, **10**(6), 969. DOI: 10.3390/vaccines10060969
11. *Chudakov D.B., Ryasantsev D.Yu., Tsaregorotseva D.S., Kotsareva O.D., Fattakhova G.V., Svirshchevskaya E.V.* (2020) Tertiary lymphoid structure related B-cell IgE isotype switching and secondary lymphoid organ linked IgE production in mouse allergy model. BMC Immunology, **21**(1), 45. DOI: 10.1186/s12865-020-00376-7
12. *Xu Z., Zan H., Pone E.J., Mai T., Casali P.* (2012) Immunoglobulin class-switch DNA recombination: Induction, targeting and beyond. Nat. Rev. Immunol., **12**(7), 517-531. DOI: 10.1038/nri3216
13. *Labmrecht B.N., Hammad H.* (2014) Allergens and the airway epithelium response: Gateway to allergic sensitization. J. Allergy Clin. Immunol., **134**(3), 499-507. DOI: 10.1016/j.jaci.2014.06.036
14. *Chan B.C.L., Lam C.W.K., Tam L.-S., Wong C.K.* (2019) IL33: Roles in allergic inflammation and therapeutic perspectives. Front. Immunol., **10**, 364. DOI: 10.3389/fimmu.2019.00364
15. *Nakada E.M., Bhakta N.R., Korwin-Michavics B.R., Kumar A., Chamberlain N., Bruno S.R., Chapman D.G., Hoffman S.M., Daphtary N., Aliyeva M., Irvin C.G., Dixon A.E., Woodruff P.G., Amin S., Poynter M.E., Desai D.H., Anathy V.* (2019) Conjugated bile acids attenuate allergen-induced airway inflammation and hyperresponsiveness by inhibiting UPR transducers. JCI Insight., **4**(9), E98101. DOI: 10.1172/jci.insight.98101
16. *Siddesha J.M., Nakada E.M., Michavicks B.R., Hoffman S.M., Rattu G.K., Chamberlain N., Cahoon J.M., Lahue K.G., Daphtary N., Aliyeva M., Chapman D.G., Desai D.H., Poynter M.E., Anathy V.* (2016) Effect of a chemical chaperone, tauroursodeoxycholic acid, on HDM-induced allergic airway disease. Am. J. Physiol. Lung Cell Mol. Physiol., **310**(11), 1243-1259. DOI: 10.1152/ajplung.00396.2015
17. *Uppala J.K., Gani A.R., Ramaiah K.V.A.* (2017) Chemical chaperone, TUDCA unlike PBA, mitigates protein aggregation efficiently and resists ER and non-ER stress induced HepG2 cell death. Sci. Rep., **7**(1), 3831. DOI: 10.1038/s41598-017-03940-1
18. *Tsukamoto Y., Uehara S., Mizoguchi C., Sato A., Horikawa K., Takatsu K.* (2005) Requirement of 8-mercaptoguanosine as a costimulus for IL-4-dependent μ to γ1 class switch recombination in CD38-activated B cells. Biochem. Biophys. Res. Commun., **336**, 625-633. DOI: 10.1016/j.bbrc.2005.08.141
19. *Wang Y., Shi J., Yan J., Xiao Z., Hou X., Lu P., Hou S., Mao T., Liu W., Ma Y., Zhang L., Yang X., Qi H.* (2017) Germinal-center development of memory B cells driven by IL-9 from follicular helper T cells. Nat. Immunol., **18**(8), 921-930. DOI: 10.1038/ni.3788
20. *Turner J.-E., Morrison P.J., Wilhelm C., Wislon M., Ahifors H., Renauld J.-C., Panzer U., Helmby H., Stockinger B.* (2013) IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. J. Exp. Med., **210**(13), 2951-2965. DOI: 10.1084/jem.20130071
21. *Meli A.P., Fontes G., Soo C.L., King I.L.* (2017) T follicular helper cell-derived IL-4 is required for IgE production during intestinal helminth infection. J. Immunol., **199**(1), 244-252. DOI: 10.4049/jimmunol.1700141

22. *Khaitov M.R., Gaisina A.R., Shilovskiy I.P., Smirnov V.V., Ramenskaia G.V., Nikonova A.A., Khaitov R.M.* (2018) The role of interleukin-33 in pathogenesis of bronchial asthma. New experimental data. Biochemistry (Moscow), **83**(1), 13-25.] DOI: 10.1134/S0006297918010029
23. *Zan H., Casali P.* (2013) Regulation of *Aicda* expression and AID activity. Autoimmunity, **46**(2), 86-101. DOI: 10.3109/08916934.2012.749244
24. *Castigli E., Wilson S., Scott S., Dedeoglu F., Xu S., Lam K.-P., Bram R., Jabara H., Geha R.* (2005) TACI and BAFF-R mediate isotype switching in B cells. J. Exp. Med., **201**(1), 35-39. DOI: 10.1084/jem.20032000
25. *Janssen E.*, *Ozcan E., Liadaki K., Jabara H.H., Manis J., Ullas S., Akira S., Fitzgerald K.A., Golenbock D.T., Geha R.S.* (2014) TRIF signaling is essential for TLR4-driven IgE class switching. J. Immunol., **192**(6), 2651-2658. DOI: 10.4049/jimmunol.1300909
26. *Dilidaer T., Zheng Y., Liu Z., Hu X., Zhang J., Hu L., Han M., Wang D., Li H.* (2017) Increased BAFF expression in nasal polyps is associated with local IgE production, Th2 response and concomitant asthma. Eur. Arch. Otorhinolaryngol., **274**(4), 1883-1890. DOI: 10.1007/s00405-016-4435-1
27. *Matsushita T., Fujimoto M., Echigo T., Matsushita Y., Shimada Y., Hasegawa M., Takehara K., Sato S.* (2008) Elevated serum levels of APRIL, but not BAFF, in patients with atopic dermatitis. Exp. Dermatol., **17**(3), 197-202. DOI: 10.1111/j.1600-0625.2007.00642.x

Received: 28. 10. 2023.
Revised: 18. 12. 2023.
Accepted: 19. 12. 2023.

ХИМИЧЕСКИЙ ШАПЕРОН TUDCA ИЗБИРАТЕЛЬНО ИНГИБИРУЕТ ПРОДУКЦИЮ АЛЛЕРГЕН-СПЕЦИФИЧЕСКИХ IgE В НИЗКОДОЗОВОЙ МОДЕЛИ АЛЛЕРГИИ

Д.Б. Чудаков*, О.А. Шустова, О.Д. Коцарева, А.А. Генералов, М.С. Стрельцова, Ю.Д. Вавилова, Г.В. Фаттахова

Государственный научный центр Институт биоорганической химии
им. акад. М.М. Шемякина и Ю.А. Овчинникова,
117997, Москва, ул. Миклухо-Маклая, 16/10; * эл. почта: boris-chudakov@yandex.ru

Клеточный ответ на стресс эндоплазматического ретикулума (ЭПР) сопровождает созревание плазматических клеток и является одним из триггеров и кофакторов локальной воспалительной реакции. В качестве средств терапии связанных со стрессом ЭПР патологий предлагаются химические шапероны — низкомолекулярные вещества, устраняющие патологический стресс ЭПР. Целью работы была оценка эффекта и механизмов влияния химических шаперонов на гуморальный ответ в низкодозовой модели аллергии. Аллергический иммунный ответ индуцировали у мышей линии BALB/c путём многократного введения овальбумина в дозе 100 нг в течение 6 недель. Части животных вместе с антигеном вводили химические шапероны TUDCA (тауроурсодезоксихолевая кислота) и 4-PBA (4-фенилбутират). Введение TUDCA, но не 4-PBA, подавляло продукцию специфического IgE (уменьшение титров в 2,5 раза). Ни один из химических шаперонов не влиял на продукцию специфических IgG_1. Эффект TUDCA был связан с подавлением переключения на синтез IgE в региональных лимфатических узлах. Данное явление было связано с подавлением экспрессии генов цитокинов 2 типа иммунного ответа, особенно *Il4* и *Il9*, что в свою очередь могло быть вызвано подавлением высвобождения IL-33. Кроме того, TUDCA достоверно подавляла экспрессию цитокина APRIL, и в меньшей степени — BAFF. Таким образом, ингибирование TUDCA продукции специфического IgE обусловлено подавлением высвобождения IL-33 и уменьшением продукции цитокинов 2 типа иммунного ответа, а также подавлением экспрессии цитокинов APRIL и BAFF.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: химический шаперон; тауроурсодезоксихолат; IgE; интерлейкин 4; интерлейкин 33; APRIL; BAFF

Финансирование. Исследование выполнено при поддержке проекта РНФ № 23-25-10044 и поддержке Бюджета города Москвы.

Поступила в редакцию: 28.10.2023; после доработки: 18.12.2023; принята к печати: 19.12.2023.