IL-4 induces ICAM-1 expression in human bronchial epithelial cells and potentiates TNF-α

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IL-4 induces ICAM-1 expression in human bronchial epithelial cells and potentiates TNF-α. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L58–L64, 1999.—Interleukin (IL)-4 is thought to contribute to the Th2 type of immune response and hence the development of allergic reactions such as asthma. In asthmatic patients, the airway epithelium expresses increased amounts of the cell surface adhesion molecule intercellular adhesion molecule (ICAM)-1 (CD54). One cytokine capable of inducing ICAM-1 in airway epithelial cells, tumor necrosis factor-α (TNF-α), is present in asthma. This study evaluated if IL-4 either alone or together with TNF-α costimulation might modulate CD54 expression by human bronchial epithelial cells (HBECs). CD54 positivity increased in response to IL-4 (16 ± 2% positive vs. 3 ± 1%, P < 0.01); greater induction of CD54 resulted from TNF-α (45 ± 2%, P < 0.001). Costimulation with TNF-α plus IL-4 further augmented expression (56 ± 1%, P < 0.05). Immunoperoxidase results were confirmed by flow cytometry. RT-PCR revealed no increase in ICAM-1 mRNA expression under control conditions or after stimulation with IL-4 alone. TNF-α increased IL-4 mRNA, and IL-4 potentiated this. Functionally, IL-4 augmented the adhesion of THP-1 monocyte/macrophage cells to monolayers of HBECs both alone and in the presence of TNF-α. We conclude that 1) IL-4 augments epithelial cell ICAM-1 expression, 2) IL-4 potentiates the adhesion of THP-1 monocyte/macrophage cells to epithelial cells, and 3) modulation of epithelial cell ICAM-1 expression by IL-4 may play a role in the immunopathology of bronchial asthma.

interleukin-4; interleukin-10; interleukin-13; intercellular adhesion molecule-1; tumor necrosis factor-α

THE EXPRESSION of intercellular adhesion molecule-1 (ICAM-1, CD54) on the membrane of bronchial epithelial cells is thought to be involved in leukocyte trafficking and activation during bronchial inflammation (7). Previous studies have shown upregulation of ICAM-1 molecules on the bronchial epithelium of asthmatics in comparison with that in normal subjects (6, 46). Because blockade of ICAM-1 can attenuate inflammatory responses (47), the increased ICAM-1 in asthma appears to have an important role in asthma pathogenesis. In vitro, several cytokines, including tumor necrosis factor (TNF)-α, which is reported to be increased in asthma (10, 19), have been demonstrated to upregulate ICAM-1 expression by the airway epithelium (1).

In asthma, Th2 lymphocytes are prominent in the airways and are thought to drive the immunopathology of the disease. In this regard, interleukin (IL)-4 hyperproduction is a prominent feature in the cytokine profile of atopic asthmatic subjects (37). This multifunctional cytokine can drive the development of Th2 cells (39) and inhibit Th1-type effector functions (34). It can also function as a switch factor for IgG1 and IgE production by B cells (35). Regarding ICAM-1 expression, a stimulatory effect of IL-4 has been found in mast cells (43) and fibroblasts (36), but minimal effects have been reported on endothelial cells (44).

The purpose of the present study was to evaluate the hypothesis that IL-4 could modulate ICAM-1 expression of human bronchial epithelial cells (HBECs) either directly or by modulating the responsiveness of the cells to TNF-α. In addition, the activity of IL-4 was compared with the related Th2-derived cytokines IL-10 and IL-13.

METHODS

Preparation of HBECs. HBECs were obtained from a donor undergoing bronchoscopy for evaluation of a lung mass with a modification of the method of Kelsen et al. (24). Informed consent was obtained in agreement with a protocol approved by the Institutional Review Board for the Protection of Human Subjects at the University of Nebraska Medical Center. After premedication with meperidine and atropine and local anesthesia with lidocaine, the flexible fiber-optic bronchoscope (Olympus model T or equivalent) was introduced into a proximal main stem or lobar bronchus. The brushing was performed in an area of normal-appearing mucosa under direct visual guidance. The brush was then retracted, and dissociated cells were recovered by vortexing the brush in ice-cold MEM for several seconds. The harvested cell suspension was filtered through a 100-µm Nitex filter (Tetko, Elmsford, NY) to remove mucus and then was treated for 10 min with 50 µg/ml DNase to eliminate clumping. Finally, the cell pellet was resuspended in culture medium, cell number was determined using a hemocytometer, and cell viability was assessed by trypan blue exclusion. For most of the experiments, cells from a single donor were used. Cells were also prepared from a second individual with a modification of the method of Lee et al. (26) using tissue harvested at rapid autopsy (25).

Cell culture techniques. Cells were cultured under serum-free conditions as described in detail previously (5) using a 1:1 mixture of RPMI 1640 medium (GIBCO BRL, Grand Island, NY) and LHC-9 (prepared from LHC basal; Biofluids, Rockville, MD). Bovine pituitary extract for supplementation of
LHC-9 was prepared as described (8). Cells were plated on Vitrogen 100 (Collagen, Palo Alto, CA)-coated tissue culture dishes at 37°C in a humidified atmosphere of 5% CO₂, and culture media were changed every 1–3 days as indicated. Second- or third-passage cells were harvested by brief trypsinization (0.25% trypsin-EDTA; GIBCO BRL), washed two times in Hanks’ balanced salt solution (HBSS), and stored in liquid nitrogen until used. Frozen cells were gently thawed at 37°C, resuspended in LHC-9-RPMI 1640 medium, and directly plated in LHC9-RPMI 1640 medium as described above. Cells were then grown as needed, taking care to passage cells before they reached dense confluency. Cells from passages 4–7 were used for experiments after the viability (>97%) was checked; purity of cultures was routinely assessed with anti-human cytokeratin antibody (MAK-6; Triton, Alameda, CA) staining, which is highly specific for cells of epithelial origin. At the time of initial isolation, >95% of cells stained positively for cytokines.

This technique of cell preparation permits the routine reevaluation of identical cells. Because there is some variability among strains of cells prepared from different individuals, the majority of experiments performed in the current study were done using a single strain of cells isolated from a normal-airway airway of an individual with carcinoma of the lung. For confirmation purposes, selected experiments were performed with additional cell strains as indicated.

Cytokine stimulation. Cells were allowed to grow to near confluence after which the culture medium LHC9-RPMI 1640 was removed and changed to growth factor-depleted LHCD-RPMI 1640 medium (LHCD-RPMI) to exclude the effect of supplements. Cytokines were dissolved in LHCD-RPMI. Cytokines used included IL-4 (Genzyme, Cambridge, MA), IL-10 and IL-13 (both PeproTech, Rocky Hill, NJ), and TNF-α (R&D Systems, Minneapolis, MN). After a 24-h exposure to cytokines, cells were harvested by a method that had proved not to affect immunoperoxidase staining for ICAM-1 expression. Briefly, cells were washed one time with LHCD-RPMI and briefly exposed to 0.25% trypsin-EDTA (GIBCO BRL). After cell detachment, which could be observed visually, the cells were harvested, and trypsin was inhibited by a 0.02% soybean trypsin inhibitor (GIBCO BRL). Cells were then washed in LHCD-RPMI and used for further experiments.

Immunoperoxidase staining of ICAM-1. Immunocytochemical analysis for the determination of the percentage of CD54-positive cells was done with an immunoperoxidase slide assay as described in detail (12) with minor modification. Briefly, 10 µl of cell suspensions (2 × 10⁵ cells/ml) were transferred to reaction areas of adhesive slides (Bio-Rad), and after attachment, cells were fixed with 0.05% glutaraldehyde. Cells were then washed with 8 g of NaCl, 0.4 g of KCl, and 1 M HEPES, pH 7.4, in 1,000 ml of distilled water (NKH buffer) and were preincubated with gelatin-blocking medium to prevent Ig binding to the glass surface. The staining procedure included the following steps: 1) 5-min incubation with anti-CD54 monoclonal antibody 8H110 (Amac) diluted 1:50 in gelatin-blocking medium; 2) 5-min incubation with rabbit anti-mouse Ig (Dako); 3) 5-min incubation with swine anti-rabbit Ig (Dako); 4) 5-min incubation with peroxidase-anti-peroxidase complex from rabbit (Dako); all antibodies were diluted 1:30 in NKH buffer; and 5) incubation with aminoethylcarbazole (Sigma) for 10 min. To evaluate the reaction, the slides were viewed under a light microscope, and a positive reaction was denoted by the presence of red, granular staining of the cell membrane. As an isotype-identical control, monoclonal antibody IOM2 (Amac) was used; the antibody showed a negative reaction with HBECs and a strongly positive reaction with peripheral blood monocytes.

Quantitative indirect immunofluorescence analysis. For a quantitative evaluation of ICAM-1 density on the membrane of stimulated HBECs, flow cytometry was used as previously described (14). HBECs obtained by gentle trypsinization were washed in PBS containing 0.02 mM sodium azide and 1% BSA and were incubated with a monodonal antibody for human ICAM-1 diluted 1:50 in PBS for 30 min on ice. After two washes, the cells were incubated with FITC-labeled goat anti-mouse antibody (Becton Dickinson), 1:50 in PBS, for 30 min on ice. After two additional washes, cells were resuspended in formaldehyde-containing fixative medium and were stored at 4°C in the dark until flow cytometry analysis by fluorescence-activated cell sorter (Becton Dickinson, Sunnyvale, CA).

mRNA analysis. Total cellular RNA was extracted from adherent HBECs cultured in six-well culture plates (Falcon, Lincoln Park, NJ) using a modification of the method of Chomczynski and Sacchi (11). After the cells were washed with HBSS, they were solubilized in 4 M guanidium thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. The resulting cell lysate was acidified with sodium acetate, pH 4.0, and extracted with phenol and chloroform-isopentyl alcohol. After precipitation with isopropanol, the resulting pellet was washed with ethanol, dissolved in 0.5% SDS, and stored at −70°C until use.

RT-PCR of ICAM-1 mRNA. Total cellular RNA (1 µg) was denatured at 95°C for 5 min and incubated at 42°C for 60 min in 20 µl of mixture consisting of 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 2.5 mM MgCl₂, 1 µg/ml RNAse (RNA-Guard; Pharmacia, Piscataway, NJ), 100 µM random hexamer (Pharmacia, 1 µM each dATP, dCTP, dGTP, and dTTP (Perkin-Elmer, Norwalk, CT), and 200 units Superscript RT enzyme (GIBCO BRL). For each reaction mixture, 2 µl of the RT product were added to PCR buffer (1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris·HCl, pH 8.3, and 0.01% gelatin; Perkin-Elmer) along with 1 µM each primer, 1 µM each dATP,

![Fig. 1. Concentration-dependent effect of interleukin (IL)-4 on intercellular adhesion molecule (ICAM)-1 expression on human bronchial epithelial cells (HBECs). HBECs were cultured in 6-well tissue culture plates until reaching confluence and then were stimulated with increasing concentrations of IL-4 (0.1–20 ng/ml) in the presence or absence of tumor necrosis factor-α (TNF-α; 5 ng/ml). After 24 h, cells were recovered by a gentle trypsinization and were stained for ICAM-1 expression by the immunoperoxidase slide assay. IL-4 increased CD54 expression in a concentration-dependent manner, reaching a peak at 5 ng/ml. The effect of IL-4 in combination with TNF-α on the induction of CD54 showed a similar concentration dependence. LHCD, growth factor-depleted LHC9.](http://ajplung.physiology.org/) by 10.220.32.246 on November 6, 2017
Table 1. Effect of cytokines on intercellular adhesion molecule-1 expression

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>n</th>
<th>% of CD54 Cells</th>
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<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>13.4 ± 1.6</td>
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<tr>
<td>IL-1</td>
<td>12</td>
<td>25.9 ± 3.9</td>
<td>0.01</td>
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<td>IL-10</td>
<td>9</td>
<td>13.4 ± 2.7</td>
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<tr>
<td>IL-13</td>
<td>9</td>
<td>19.9 ± 4.6</td>
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<tr>
<td>TNF-α</td>
<td>12</td>
<td>54 ± 7.5</td>
<td></td>
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<tr>
<td>TNF-α IL-1</td>
<td>6</td>
<td>68.3 ± 4.1</td>
<td>0.05</td>
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<tr>
<td>TNF-α IL-10</td>
<td>6</td>
<td>49 ± 2.1</td>
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<tr>
<td>TNF-α IL-13</td>
<td>6</td>
<td>57.7 ± 3.0</td>
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Values are means ± SE; n, number of individual experiments. IL, interleukin; TNF, tumor necrosis factor.

Macrophage attachment assay. After reaching confluency, HBEcs cultured in 96-well tissue culture plates (Falcon) were exposed to cytokines for 24 h. Cells were then washed and cocultured for 15 min with $0.3 \times 10^6$ THP-1 monocyte/macrophage cells (American Type Culture Collection, Manassas, VA) per well. These cells had been cultured in RPMI 1640 medium supplemented with 10% FCS and $5 \times 10^{-5}$ M 2-mercaptoethanol. Before attachment assay, THP-1 cells were labeled with the fluorescent dye '2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF; Molecular Probes, Eugene, OR; see Ref. 13). After the incubation, the top of the plate was hermetically covered, the plate was gently inverted, and nonadherent cells were sedimented for 5 min. All wells were then gently washed with MEM and HBSS (phenol red free). Attached cells were solubilized with 0.3% Triton in distilled water. The cell lysate was then transferred to a 96-well Microfluor plate (Dynatech, Chantilly, VT) and evaluated by an automatic microfluorometer Fluoroscan (Perkin-Elmer) at 485/535-nm excitation/emission wavelengths.

Statistics. Nonparametric statistics, the Kruskal-Wallis and Mann-Whitney tests, were used to compare groups and paired data, respectively. Spearman's rank correlations were used for the statistical analysis of differences in the membrane expression of CD54 on cytokine-stimulated cells and for the changes in the attachment rate of THP-1 cells to HBEcs monolayers. P values below 0.05 were considered significant.

RESULTS

Modulation of HBEc ICAM-1 expression by IL-4. IL-4 increased the percentage of HBEcCs that expressed ICAM-1 in a concentration-dependent manner (Fig. 1).
At the maximum dose tested, ~40% of HBECs expressed ICAM-1, a percentage similar to that induced by 5 ng/ml TNF-α. When IL-4 was added in the presence of 5 ng/ml TNF-α, it further augmented HBEC ICAM-1 expression (Fig. 1). For a comparison, we also evaluated IL-4 together with single concentrations of other Th2-related cytokines, IL-13 and IL-10, to induce ICAM-1 both alone and in the presence of TNF-α (Table 1). Although there is more variability between experiments than within an experiment, results are consistent (Table 1). IL-13 has been shown to stimulate ICAM-1 expression by HBECs similarly to IL-4; however, it does not potentiate the effect of TNF-α in these cells.

To confirm the results obtained by visual scoring, flow cytometric analysis was performed and yielded results similar to those obtained by direct counting (Fig. 2). Compared with unstimulated HBECs (Fig. 2A), cells cultured in the presence of IL-4 (Fig. 2B) and TNF-α (Fig. 2C) demonstrated increased staining. The combination of IL-4 and TNF-α further augmented staining (Fig. 2D). The quantitative data are expressed in Fig. 3; both the mean fluorescence intensity and the median value (in parentheses) increased from 49 (21) for unstimulated cells to 93 (34) for IL-4-, to 297 (195) for TNF-α-, and to 489 (308) for IL-4- and TNF-α-stimulated cells.

ICAM-1 mRNA expression in IL-4- and TNF-α-stimulated HBECs. Using the more sensitive method of RT-PCR, constitutive levels of ICAM-1 mRNA were demonstrable, and no increase was observed after IL-4 exposure (Fig. 4). TNF-α exposure induced readily detectable ICAM-1 mRNA, and IL-4 augmented this.

Attachment of THP-1 monocytes/macrophages to HBECs. To assess if the increased ICAM-1 expression was associated with altered function of the HBECs, binding of the monocyte/macrophage cell line THP-1 was measured. Binding of BCECF-labeled THP-1 cells to HBECs (Fig. 5) increased significantly when the epithelial monolayer was preincubated with IL-4 (146 ± 1% of control, P = 0.03) or TNF-α (152 ± 1%, P = 0.01). The combination of both cytokines further increased attachment of THP-1 cells (202 ± 10% of control, P = 0.012). The absolute values of fluorescence intensity are presented in Fig. 5. The anti-ICAM-1 antibody 84H10 partially inhibited THP-1 binding under all conditions (Fig. 6).

Effects of other Th2 cytokines. At a concentration of 10 ng/ml, at which the effect of IL-4 was maximal, IL-13 stimulated ICAM-1 expression slightly and IL-10 was without effect (Table 1). Neither IL-10 nor IL-13, however, altered the TNF-α-induced expression of ICAM-1. Thus the effects of IL-4 differed markedly from those of IL-10 and IL-13.

DISCUSSION

In addition to airway hyperreactivity, asthma is characterized by the presence of activated immune cells in the airway epithelium (4, 21). Lymphocytes
producing cytokines IL-4, IL-5, and IL-13, called Th2 according to a previously described murine nomenclature, are thought to play a particularly important role (2). These cells are prominent in the asthmatic airway epithelium, and cytokines released by these cells can drive both eosinophil accumulation and B-cell production of IgE. The epithelial cells that line the airways are also involved in the immune response, both producing cytokines (38) and expressing increased amounts of cell surface adhesion receptors for inflammatory cells (18, 23, 41, 46, 47). The present study suggests that Th2 cells, through the production of IL-4, may also drive this aspect of the immune response in asthma.

Increased expression of the cell surface adhesion receptor ICAM-1 has been demonstrated by immunohistochemistry in asthma (18, 46). This increased expression is accompanied by increased mRNA expression as demonstrated by in situ hybridization. Several media tors have been reported to increase ICAM-1 expression in vitro including culture of human airway epithelial cells, including histamine (45) and the Th1- and inflammatory-derived cell cytokines TNF-α, IL-1β, and interferon-γ (30). Although histamine may play a role in asthma, Th1 cells are generally felt to be lacking in asthma and to have the potential to suppress an asthmatic Th2-type response. The present study demonstrates that Th2 cytokines can also upregulate epithelial cell ICAM-1. Moreover, IL-4 and TNF-α, a cytokine released by Th2 as well as Th1 cells, can interact cooperatively to further increase ICAM-1 expression. Activation of Th2 lymphocytes in the airway epithelium, therefore, may drive not only the eosinophil and the B-cell response but may also contribute to the epithelial changes characteristic of asthma. We have derived our data using cells from nonatopic and nonasthmatic subjects; nevertheless, further studies with cells from airway brushings of patients with bronchial asthma would be of potential interest. Such studies may reveal either an acquired or a genetically based difference in cytokine induction of ICAM-1 by epithelial cells.

Under control conditions, no detectable ICAM-1 mRNA was detected in the present study, presumably because levels were below the limit of detectability. This result agrees with both the in vitro studies of Look et al. (30), who were also unable to detect ICAM-1 mRNA, and the in situ hybridization studies of normal airway epithelium of Vignola et al. (46), who detected no ICAM-1 mRNA. Despite the inability to detect mRNA, faintly detectable surface expression of ICAM-1 was observed in a small percentage of control cells. Although IL-4 increased surface expression, mRNA was not increased. TNF-α, in contrast, markedly increased ICAM-1 mRNA. IL-4 together with TNF-α potentiated the increased ICAM-1 mRNA expression in parallel with its effect on surface molecule expression.

The IL-4 and IL-13 genes share a common intron-exon structure on chromosome 5q, and the degree of homology between IL-4 and IL-13 protein sequences is ∼30% (48). IL-13 shares many of the known activities of IL-4 on monocytes. Both cytokines induce morphological transformation of monocytes into cells of dendritic appearance (15), downregulate CD14 and Fc receptors, and upregulate the expression of class II major histocompatibility (MHC) antigens (16). Both cytokines inhibit proinflammatory cytokine release (20, 32), ni-
tric oxide production (28), and killing of intracellular parasites (40). IL-4 and IL-13 also have similar activities on B cells, inducing their proliferation, differentiation, and Ig switching to the synthesis of IgE (3). However, IL-13 fails to activate T cells in contrast to the growth-promoting effect of IL-4 (48). Our data suggest another unique activity of IL-4 compared with that of IL-13, since IL-4 augments TNF-α-induced expression of ICAM-1 by HBECs, but IL-13 does not. It remains possible, of course, that IL-13 may exert such effects but may be much less potent in this regard than IL-4.

IL-10 is another Th2-derived cytokine with some effects that resemble IL-4. IL-10 is a potent immunosuppressant of macrophage function, although it augments both the proliferation and differentiation of B cells (33). Whereas there are several IL-4-like effects of IL-10, this cytokine has been found to inhibit the upregulation of class II MHC molecules on monocytes induced by IL-4 (17). Also, the effects of IL-10 on NK cells differ from those of IL-4 in that IL-4 inhibits their activity, whereas IL-10 enhances their interferon production and lymphokine-activated killer activity (22). We found a very mild inhibitory effect of IL-10 on ICAM-1 induction by HBECs in contrast to a clear stimulatory effect of IL-4.

Adhesion of human leukocytes to the airway epithelium is mediated by both ICAM-1-dependent and ICAM-1-independent mechanisms (42). Upregulation of ICAM-1 surface expression was accompanied by increased binding of THP-1 cells, suggesting that increased expression altered the functional activity of the HBECs. However, anti-ICAM-1 antibody caused only partial inhibition of TNF-α-monocytes/macrophages attachment to HBECs in both unstimulated and cytokine-induced cells. Our incomplete inhibition of TNF-α binding with anti-ICAM-1 antibody is in agreement with similar blocking experiments of others (42) and suggests multiligand-mediated interactions between leukocytes and epithelial cells. Thus we cannot exclude that, in addition to IL-4-mediated increase of ICAM-1 expression, this cytokine also induces increased expression of other adhesion receptors on the epithelial cell surface.

Our results in which IL-4 and TNF-α interacted to increase THP-1 binding are also consistent with the animal model studies of Leung et al. (27). In these studies, IL-4 and TNF-α interacted to increase both cell surface adhesion receptor expression and differentiation of a myeloid cell line. Although the functional significance of epithelial cell ICAM-1 expression in asthma is unknown, some evidence suggests that it may play a pathogenetic role and therefore may be a therapeutic target. Specifically, ICAM-1 blockade by specific antibodies has been shown to attenuate the symptoms of experimental bronchial hypersensitivity induced in primates (47). A possible link between cytokines derived from Th2 cells and the induction of adhesion molecules on the airway epithelium suggests an alternative strategy to affect epithelial cell ICAM-1 expression therapeutically. In this regard, anti-IL-4 therapy using soluble IL-4 receptors has been recently tested in animal models of inflammation (31).

In summary, IL-4 stimulates HBEC expression of ICAM-1 (CD54). IL-4 also potentiates the induction of ICAM-1 induced by TNF-α. Of the IL-4-related cytokines, IL-13 also induced ICAM-1 expression, whereas IL-10 lacked this activity, and neither potentiated TNF-α-induced ICAM-1 expression. The ability of Th2-derived cytokines to induce the expression of ICAM-1 on airway epithelial cells can lead to increased adhesion of inflammatory cells. This pathway may contribute to the pathogenesis of bronchial asthma, a condition in which both Th2-derived cytokines and TNF-α are present in high concentrations.

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