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Antibodies to C-C Chemokine Receptor 5 in Normal Human IgG Block Infection of Macrophages and Lymphocytes with Primary R5-Tropic Strains of HIV-1

Hicham Bouhlal,* Hakim Hocini,* Caroline Quillec-Grégoire,† Vladimira Donkova,* Stephanie Rose,* Ali Amara,* Renato Longhi,* Nicole Haeffner-Cavaillon,* Alberto Beretta,*† Srini V. Kaveri,∗ and Michel D. Kazatchkine*

In the present study, we demonstrate that normal human IgG for therapeutic use (i.v. Ig) contains natural Abs directed against the CCR5 coreceptor for HIV-1. Abs to CCR5 were isolated from i.v. Ig using an affinity matrix consisting of a synthetic peptide corresponding to the N-terminus of CCR5 coupled to Sepharose. Natural anti-CCR5 Abs inhibited the binding of RANTES to macrophages, demonstrating their interaction with the coreceptor of R5-tropic HIV-1. Affinity-purified anti-CCR5 Ig further inhibited infection of lymphocytes and monocytes/macrophages with primary and laboratory-adapted strains of HIV-1, but did not inhibit infection with X4-tropic HIV. Our results suggest that anti-CCR5 Abs from healthy immunocompetent donors may be suitable for development of novel passive immunotherapy regimens in specific clinical settings in HIV infection. 

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ormal human serum contains natural Abs of the IgG, IgM, and IgA isotypes that are produced in the absence of deliberate immunization and independently of exposure to foreign Ags (1). Most natural Abs in the serum of healthy individuals are self-reactive (natural autoantibodies (NAAbs))3. Recent evidence substantiates the hypothesis that NAAbs are generated by positively selected autoreactive B cells (2). Several functions have been proposed for NAAbs, including a role in natural host defense against infection and in the control of immune homeostasis (3). The role of NAAbs in immune regulation has been documented by studies of the effects of i.v. IgG therapy in patients with autoimmune diseases, i.v. Ig containing large amounts of natural IgG Abs obtained from pools of plasma of several thousand healthy blood donors. Several of the postulated mechanisms of action of i.v. Ig relate to the presence in i.v. Ig of Abs to molecules of relevance for regulation of the immune response. Thus, i.v. Ig has been shown to contain Abs directed against several cell surface molecules, including CD4, CD5, cytokine receptors, adhesion motifs, and CD95 (4).

CXCX4 and CCR5 chemokine receptors function as the major coreceptors for T lymphocyte and macrophage-tropic HIV-1 isolates, also referred to as X4 and R5 isolates, respectively (5, 6). R5-tropic strains account for most sexually transmitted HIV infections (7). The importance of CCR5 for HIV-1 transmission was evidenced by the observation that individuals homozygous for a defective CCR5 allele remained uninfected despite repeated exposure to HIV (8–12). The CCR5 molecule is thus being considered as a target for therapeutic strategies designed to block HIV-1 entry into cells (13).

In the present study, we demonstrate that i.v. Ig contains Abs that recognize a CCR5 peptide motif corresponding to the N-terminus of CCR5 (II.E/C-CCR5) molecule. Affinity-purified anti-CCR5 Abs bound to the CCR5 molecule expressed on transfected Chinese hamster ovary (CHO) cells and on monocyte-derived macrophages (MDM) and blocked the binding of RANTES to its receptor. Anti-CCR5 Ig further inhibited the infection of monocytes/macrophages and lymphocytes by both primary and laboratory-adapted R5-tropic HIV-1 strains.

Materials and Methods

Abs and reagents

Intravenous Ig (Sandoglobulin) was a gift from the Central Laboratory of the Swiss Red Cross (Bern, Switzerland). F(ab′)2 were prepared from i.v. Ig by pepsin digestion (2% w/w; Sigma, St. Louis, MO) in acetate buffer, pH 4.1, for 18 h at 37°C, followed by chromatography on protein G-Sepharose. F(ab′)2 were free of intact IgG and Fc fragments, as assessed by SDS-PAGE and ELISA. FITC-conjugated mAb to CD4 (Leu-3a) was purchased from BD Biosciences (Le Pont de Claiix, France). PE-conjugated mAb to CCR5 (clone 2D7) and PE-conjugated mAb to human RANTES (clone 2D5) were obtained from PharMingen (Le Pont de Claiix, France). The PE cyanin5-conjugated anti-CD14 (ROM52) was obtained from Immunotech Beckman Coulter (Villepinte, France). Biotinylated goat anti-mouse IgG and PE-conjugated goat anti-human IgG were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). The chemokines RANTES, SDF-1, and IL-2 were obtained from R&D Systems Europe (Abingdon, Oxon, U.K.). PHA was obtained from Sigma, and the CCR5 reagent was obtained from Boehringer Mannheim (Meylan, France).

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2 Address correspondence and reprint requests to Dr. Srini Kaveri, Institut National de la Sante et de la Recherche Medicale Entite II 430, Hopital Broussais, 96 rue Didot, 75674 Paris Cedex 14, France. E-mail address: srini.kaveri@brs.ap-hop-paris.fr

3 Abbreviations used in this paper: NAAbs, natural autoantibodies; CHO, Chinese hamster ovary; CCR5, chemokine receptor-chemokine receptor-5; F(ab′)2, fragment (Fab′)2; PE, phycoerythrin; hRANTES, human recombinant RANTES; MDM, monocyte-derived macrophage; SDF, stromal-derived factor.
The peptide CCR5 (MDYQVSPSIIYDNYTSEPC) that corresponds to the N-terminus of CCR5 was synthesized by the solid-phase F-moc method using an Applied Biosystems model 433A peptide synthesizer (Foster City, CA) (14).

**Immunoaffinity purification of anti-CCR5 Abs**

For affinity purification of anti-CCR5 Ig, the CCR5 peptide (20 aa) was coupled to activated CH Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden), and the column was equilibrated with PBS, pH 7, before being loaded with 30 mg of i.v. Ig per 2 ml of matrix containing 3 mg of the peptide. i.v. Ig was allowed to interact with the matrix overnight at 4°C before washing the column with PBS, pH 7, and elution with glycine HCl buffer (0.2 mol/L, pH 2.8). Eluted anti-CCR5 Ig was neutralized with 3 mol/L Tris and dialyzed against PBS overnight. F(ab')2 of i.v. Ig were also subjected to affinity chromatography in a similar fashion.

**Binding studies of anti-CCR5 Ig**

The reactivity of affinity-purified anti-CCR5 Ig with the CCR5 peptide was assessed using an ELISA. Plates were coated with 50 μL of CCR5 peptide (10 μg/ml) in PBS, pH 7.4, for 1 h at 37°C. The wells were washed with PBS/Tween (1) before saturation with PBS-BSA 0.1%. Several dilutions of immunopurified anti-CCR5 Ig were added (100 μl) for 2 h at 37°C. Positive and negative controls consisted of mouse anti-CCR5 mAb 2D7 (10 μg/ml) and of the eluent of the affinity column (anti-CCR5-depleted IgG; 200 μg/ml), respectively. After further washing with PBS-Tween, biotinylated goat anti-mouse IgG, goat anti-human IgG, or goat anti-human F(ab')2 were added for 1 h at 37°C. Following an incubation step with streptavidin, substrate was added and the OD was recorded at 490 nm.

**Primary cells and cell lines**

The CCR5-GFP-CHO are CHO cells transfected with human CCR5 cDNA fused to a green-fluorescent protein (GFP) vector that encodes a neomycin-resistant gene (15). CCR5-GFP-CHO cells were cultured in Ham’s F12 medium containing FCS (10%) and antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; and neomycin G418, 800 μg/ml) from Life Technologies (Rockville, MD). HeLa-CD4-CCR5-LTR/α2 cells (P4-CCR5) were maintained in DMEM medium containing FCS (10%), penicillin (100 U/ml), streptomycin (100 μg/ml), and puromycin (1 μg/ml). Lymphocytes were obtained by stimulation of PBMC of HIV-1-seronegative healthy individuals with PHA (2.5 μg/ml) for 72 h before stimulation with IL-2 (10 IU) for 24 h. Monocytes/macrophages were obtained by culturing PBMC in RPMI 1640 containing FCS (10%), penicillin (100 U/ml), streptomycin (100 μg/ml), and M-CSF (200 U/ml). Monocytes were maintained for 5–7 days to obtain MDM. Purity of the MDM preparation was assessed by morphological criteria and flow cytometry analysis (80–90% CD14 positive and contamination with T cells <3%).

**Immunofluorescent staining and cytofluorimetry**

The CCR5-GFP-CHO and MDM were used to assess the specificity of interaction of purified anti-CCR5 Ig and anti-CCR5 Ig F(ab')2 with membrane-bound CCR5. CCR5-GFP-CHO and MDM were recovered in cold PBS and EDTA (0.02%) for 10 min. Cells (106 in 200 μL PBS/NaNO3 0.05%) were incubated with increasing amounts of anti-CCR5 Ig or F(ab')2 (100–500 μg/ml) for 30 min on ice. Anti-CCR5-depleted IgG was used as negative control, and anti-CCR5 mAb 2D7 as positive control. After washing, mononuclear adherent cells were cultured in RPMI 1640 containing FCS (10%), penicillin (100 U/ml), streptomycin (100 μg/ml), and M-CSF (200 U/ml). Monocytes were maintained for 5–7 days to obtain MDM. Purity of the MDM preparation was assessed by morphological criteria and flow cytometry analysis (80–90% CD14 positive and contamination with T cells <3%).

**Chemokine binding and competition assay**

CCR5-GFP-CHO cells were collected in Ca2+- and Mg2+-free PBS and 5 mmol/L EDTA, and resuspended in binding buffer PBS/BSA (0.2%)/NaN3 (0.05 M). In a first set of experiments, a saturation curve was established by incubating increasing amounts of human RANTES (hRANTES; 1–500 ng/ml) with CCR5-GFP-CHO-positive cells for 30 min. The binding of hRANTES to cells was assessed by FACS analysis using PE-labeled anti-RANTES mAb. Wild-type CHO cells were used to determine nonspecific binding. Competition experiments were then performed using 105 cells in a final volume of 200 μL PBS/BSA (0.2%)/NaN3 (0.05 M) in the presence of 10 ng/ml hRANTES and varying amounts of anti-CCR5 Ig or F(ab')2 for 30 min on ice. Unbound RANTES was eliminated by washing with PBS/BSA (0.2%)/NaN3 (0.05 M), and cells were incubated with PE-conjugated anti-RANTES for 30 min on ice. Cells were fixed with 1% paraformaldehyde in PBS. The ability of anti-CCR5 Ig and F(ab')2 to inhibit the binding of RANTES to CCR5-GFP-CHO was analyzed by flow cytometry.

**Viral strains**

Primary R5-tropic strains YU-2 and JR-CSF, and the X4-tropic strain NDK were a gift from the repository of the Center Intégré de Recherches Biomédicales sur le SIDA (Hôpital Saint Joseph, Paris, France).

**HIV-1 infection assay**

P4-CCR5 (HeLa-CD4+ CXCR4+ CCR5+) cells were infected with the laboratory-adapted HIV-1 strain BaL, the primary R5-tropic HIV-1 strains YU-2 and JR-CSF, and primary X4-tropic HIV-1 strain NDK in the presence of 200 and 500 μg/ml anti-CCR5 Ig. Briefly, cells were cultured in 96 wells (103 cells/ml) in the presence of DEAE-dextran (60 μg/ml) in DMEM/FCS (10%), penicillin (100 U/ml), streptomycin (100 μg/ml), and puromycin (1 μg/ml) for 48 h at 37°C. Cells were infected with appropriate viral dilutions and incubated for 48 h at 37°C. After washing, cells were lysed in 1 M Na2HPO4, 1 M NaH2PO4, 1 M KCl, 1 M MgSO4, 0.5 M EDTA, 50 mM 2-ME, and 10% Nonidet P-40, and CPRG staining was performed by adding the CPRG reagent to cell lysates. The OD was measured at 570 nm. PBL (2 × 106) and MDM (5 × 104) were incubated with anti-CCR5 Ig, anti-CCR5-depleted IgG as negative control (500 μg/ml), or RANTES (500 ng/ml) for 30 min at 37°C before adding virus (BaL, YU-2, and JR-CSF; 20 ng/ml per well). In some experiments, MDM were incubated with anti-CCR5 Ig at 200 and 500 μg/ml, mAb 2D7 (10 μg/ml), or SDF-1 (2.5 μg/ml) before exposure to the BaL. HIV-1 strain for 18 h at 37°C. Cells were washed three times, fresh medium was added, and supernatants were collected every 3 days. HIV-1 production was assessed by measuring p24 release using a p24 Ag capture ELISA (HIV-1 core profile ELISA; DaPond de Nemours, Les Ulis, France).

**Results**

**Intravenous Ig contains Abs reactive with CCR5**

Affinity purification of anti-CCR5 Ig yielded ~0.2% of total i.v. Ig. We first assessed immunoafluorescence-purified anti-CCR5 Abs from i.v. Ig (anti-CCR5 Ig) for reactivity with the CCR5 peptide by ELISA. Mouse mAb 2D7 that interacts with the CCR5 protein was used as positive control, and the effluent of the CCR5 affinity column containing anti-CCR5-depleted IgG was used as negative control. As shown in Fig. 1, anti-CCR5 Ig bound to the CCR5 peptide in a dose-dependent manner, whereas Ig depleted of anti-CCR5 Abs failed to recognize the peptide. Similar results were obtained using F(ab')2 of natural anti-CCR5 Abs instead of intact IgG (data not shown). As expected, anti-CCR5 mAb 2D7 bound to the CCR5 peptide (Fig. 1).

**FIGURE 1.** Binding of affinity-purified anti-CCR5 to a 20-aa peptide derived from the N terminus of CCR5. Affinity-purified anti-CCR5 Ig (C), i.v. Ig depleted of anti-CCR5 Abs (△; 25–200 μg/ml), and an anti-CCR5 mAb (2D7) (○; 1.25–10 μg/ml) were incubated with the peptide immobilized on a microtiter plate. The recognition of CCR5 motif by Ig was assessed by an ELISA, as described in Materials and Methods.
We then investigated the ability of anti-CCR5 Ig to bind to the native CCR5 molecule expressed by CCR5-CHO cells and MDM by flow cytometry. CCR5-transfected cells permanently express the fluorescence marker GFP coupled to CCR5 (90% GFP-CCR5 positive). As shown in Fig. 2, purified anti-CCR5 Ig and the corresponding F(ab')2 bound in a dose-dependent manner to CCR5-bearing cells. At 500 μg/ml, anti-CCR5 Ig and F(ab')2 recognized 81% and 57% of CCR5-positive cells, respectively, whereas the Abs did not bind to untransfected CHO cells (less than 2%; data not shown). Anti-CCR5-depleted IgG displayed marginal binding to the CCR5-CHO cells, whereas the anti-CCR5 mAb 2D7 stained 90% of the cells (Fig. 2). MDM express high level of CCR5 molecules (up to 80%). Anti-CCR5 Ig also bound to MDM in a dose-dependent manner (75% of positive cells at 500 μg/ml) (Fig. 3). A similar level of binding was observed when using the anti-CCR5 F(ab')2 fraction instead of intact anti-CCR5 Ig (data not shown). In the latter experiments, mAbs to CCR5 and to CD4 were used as positive and negative controls, respectively (data not shown).

Anti-CCR5 Ig inhibit the binding of RANTES to CCR5-expressing cells

hrRANTES bound to CCR5-CHO cells in a dose-dependent manner, and saturation was reached at 50 ng of hrRANTES, and no binding was detected when untransfected cells were used. For inhibition experiments, hrRANTES (10 ng/ml) was incubated with CCR5-CHO cells in the presence of increasing amounts of anti-CCR5 Ig or the corresponding anti-CCR5 F(ab')2 (0–1000 μg). Both anti-CCR5 Ig and anti-CCR5 F(ab')2 inhibited the binding of RANTES to CHO-CCR5 cells in a dose-dependent fashion (Fig. 2).
4. Complete inhibition was observed in the presence of 500 μg/ml of anti-CCR5 Ig. Abs depleted of anti-CCR5 IgG exhibited no inhibitory effect (Fig. 4).

Purified natural anti-CCR5 Abs block CCR5-dependent HIV infection

The HeLa P4-CCR5 cells that are CD4<sup>+</sup> CXCR4<sup>+</sup> CCR5<sup>+</sup> were infected with the BaL strain, the primary R5-tropic HIV-1 strains YU-2 and JR-CSF, or the primary X4-tropic HIV-1 strain NDK. Infection of the cells was detected using a β-galactosidase activity measured by the CPRG colorimetric test, allowing for the detection of one viral replication cycle. Inhibition experiments were performed in the presence of 200 and 500 μg/ml of anti-CCR5 Ig. As shown in Fig. 5, anti-CCR5 Ig inhibited the replication of BaL (77–81% of inhibition), of the primary R5-tropic HIV-1 YU-2, and of JR-CSF strains (60 and 64% of inhibition for YU-2 and JR-CSF, respectively). Anti-CCR5 Ig had no effect on infection of P4-CCR5 with the primary X4-tropic NDK strain. Incubation with RANTES (500 ng/ml), used as positive control, resulted in a complete inhibition of viral replication in P4-CCR5 cells infected with BaL, YU-2, and JR-CSF, whereas RANTES had no effect on infection with HIV-1 NDK. No inhibition of infection was observed in the presence of Ig depleted of anti-CCR5 Abs (Fig. 5).

We then investigated the effect of anti-CCR5 Ig on infection of MDM and PBL with HIV. MDM and PBL were incubated with anti-CCR5 Ig (500 μg/ml) and with anti-CCR5-depleted IgG before infection with the HIV-1 BaL, YU-2, and JR-CSF strains (20 ng/ml of p24). The kinetics of production of p24 in cell culture supernatants was monitored by ELISA. As shown in Fig. 6, the production of p24 by infected MDM was significantly inhibited in cultures preincubated with anti-CCR5 Ig (60% inhibition in case of BaL, 37% for YU-2, and 64% for JR-CSF) as compared with cultures incubated in the presence of anti-CCR5-depleted IgG (<5%). In another set of experiments, RANTES and anti-CCR5 mAb 2D7 inhibited up to 75% the infection of MDM by BaL, which was similar to the inhibition observed in the presence of 500 μg/ml anti-CCR5 Ig. Neither SDF-1 nor the isotypic control for mAb 2D7 had any inhibitory capacity (data not shown). Anti-CCR5 Ig also inhibited up to 90% the infection of lymphocytes with BaL, 70% in case of YU-2, and 73% in case of JR-CSF.

Discussion

We report that natural Abs directed against CCR5, the coreceptor for R5-tropic HIV-1 strains, are present in normal human IgG that inhibit the infection of human macrophages and CD4<sup>+</sup> T lymphocytes with laboratory and field R5-tropic isolates of HIV-1. We have isolated anti-CCR5 Ig from pooled normal human IgG (i.v. Ig) by affinity chromatography on a synthetic peptide that corresponds to a 20-mer motif derived from the N terminus of CCR5.
The latter region is involved in the binding of gp120 and of natural CCR5 ligands. Natural anti-CCR5 Ig recognized both immobilized CCR5 and CCR5 molecules expressed on transfected CHO cells as well as on human MDM. Affinity-purified anti-CCR5 Ig and its corresponding F(ab')2 interacted specifically with the chemokine binding site of CCR5, as demonstrated by their capability to inhibit the binding of hrRANTES to CCR5 in a dose-dependent manner. Natural anti-CCR5 Ig blocked the infection of transfected CD4+ CXCR4+ CCR5+ HeLa cells with the primary R5-tropic HIV-1 strains BaL, YU-2, and JR-CSF, but not with the primary X4-tropic NDK strain. Anti-CCR5 Ig also blocked infection with R5-tropic viral strains of macrophages and of IL-2-stimulated normal human CD4+ T lymphocytes. The inhibition achieved with anti-CCR5 Ig was comparable with that observed with the murine anti-CCR5 mAb 2D7 and with RANTES. Anti-CCR5 Ig was more effective at inhibiting the infection of lymphocytes than that of MDM, which relates to the differential expression of CCR5 on these cell types. Indeed, only 4–8% of stimulated lymphocytes expressed CCR5, as compared with 60–70% in case of MDM. In some of the viral inhibition assays, anti-CCR5-depleted Ig displayed a certain degree of viral infection. This may be due to 1) an incomplete absorption of the anti-CCR5 Ig by the affinity columns; 2) the presence in i.v. Ig of Abs directed against the other HIV coreceptors; and 3) the presence of Abs in i.v. Ig that recognize other epitopes than that presented by the peptide used to prepare the affinity matrix.

NAAbs reactive with self Ags of the IgG, IgM, and IgA isotypes are present in normal serum. NAAbs are encoded by unmutated, germline genes encoding V regions with no or few mutations. NAAbs are characteristically more polyreactive than immune Abs, in the sense that they often recognize several Ags (16). NAAbs exhibit a broad range of affinities, with dissociation constants ranging from 10^{-5} to 10^{-8} M (1, 16, 17). Using the surface plasmon resonance technology, we have observed an overall affinity in the micromolar range of natural IgG autoantibodies specific for molecules such as HLA class I, CD4, the RGD motif, and autologous blood group Ags (18–20). Several functions have been proposed for NAAbs (21), including a role in natural host defense against infection (3).

The presence of anti-CCR5 Abs has previously been documented in sera of individuals homozygous for a 32-bp deletion in the gene encoding for CCR5 (CCR5Δ32), who had been repeatedly exposed to CCR5-expressing blood cells through sexual activity (22). Abs from these individuals, in contrast to serum from CCR5Δ32 individuals, competed with radiolabeled RANTES for binding to the CCR5 receptor and inhibited infection of PBMC with R5, but not X4, primary isolates of HIV-1 (22). The role of natural anti-CCR5 Abs in vivo remains unknown. However, a recent study demonstrated that protection against HIV infection correlated with the presence of natural anti-CCR5 Abs in the sera of some HIV-exposed individuals who remain seronegative (23). The anti-CCR5 Abs down-modulated surface CCR5 expression in vivo and neutralized the infectivity of R5 strains of HIV-1, providing a basis for the acquisition of resistance to infection. R5-tropic viral strains are preferentially transmitted via the mucosal route (24, 25). After crossing epithelial barrier, virus spreads rapidly, through contact between dendritic cells and CD4+ lymphocytes cells that favor HIV replication in the early stage of infection (26–28).

Hence, blockade of CCR5 with anti-CCR5 Abs could result in the inhibition of the spreading of R5-tropic viruses in vivo. CCR5 and CXCR4, the coreceptors of R5-tropic and X4-tropic strains of HIV-1, respectively, represent a potential target to block the entry of virus and infection of lymphocytes and macrophages in vivo. A number of mAbs have been developed in attempt to block the interaction of virus with the CCR5 coreceptor (13, 29). Of note, a murine anti-CCR5 mAb that recognizes the second extracellular loop of CCR5, the major domain for gp120 binding, was shown to block the infection of T lymphocytes with several R5-tropic HIV-1 strains (30). Although murine hybridomas serve as excellent tools for understanding the structure-function relationship between HIV and its coreceptors, they bear obvious limitations from therapeutic point of view. As previously described, i.v. Ig also contain anti-CD4 Abs that block HIV entry (18). The presence of natural anti-CD4 and anti-CCR5 in i.v. Ig that blocks HIV infection allows speculation of the presence of natural Abs directed against the other HIV coreceptors, i.e., CXCR4, CCR3, and CCR2b. Thus, one may consider using i.v. Ig to prepare therapeutically relevant Abs using sequential steps of affinity chromatography.

Because i.v. Ig preparations are in clinical use, our observations suggest that affinity-purified anti-CCR5 Abs from i.v. Ig could be used therapeutically. There is a current interest in the development of anti-CCR5 antagonists for therapeutic use (31). One of the approaches could be the prevention of mother-to-child transmission...
of HIV. In this context, it is of interest that several combinations of human mAbs against HIV-1 neutralize SHIV-vpu (a chimeric simian-human virus) in vitro through synergistic interactions, and that the combination of mAbs protects macaques against i.v. SHIV-vpu challenge after delivery (32). In this respect, the identification of natural human Abs with inhibitory capacity that can be isolated from available therapeutic preparations of normal IgG emphasizes the potential of these Abs for passive immunotherapy in HIV infection.

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