

Short communication

Dexamethasone and IL-10 stimulate glucocorticoid-induced leucine zipper synthesis by human mast cells

Background: Glucocorticoids (GCs) decrease tissue mast cell (MC) number and prevent their activation via their high-affinity IgE receptor. Glucocorticoid-induced leucine zipper (GILZ) is one of the GC-induced genes, which inhibits the functions of the transcriptional activators AP-1 and NF- κ B. GILZ appears to be a critical actor in the anti-inflammatory and immunosuppressive effects of GCs in human T lymphocytes, macrophages and dendritic cells.

Aims of the study: We investigated whether GILZ was produced by human MCs and whether GILZ synthesis was stimulated by GCs. We also investigated whether GILZ production was modulated by (i) IL-10, because of its common immunosuppressive properties with GCs, (ii) histamine because of its pro-inflammatory properties and (iii) IL-4 and IL-5 because of their ability to favour MC survival and proliferation with SCF.

Methods: The human MC lines HMC-1 5C6 and LAD-2, and cord blood-derived MCs (CB-MCs) were cultured alone or in the presence of GCs, IL-10, histamine, IL-4 or IL-5. The expression of GILZ was evaluated by using RT-PCR, Western blotting or immunocytochemistry.

Results: We found that human MC lines and CB-MCs constitutively produce GILZ. We also show that GCs and IL-10 stimulate GILZ production by human MCs. Our present results indicate that histamine, IL-4 and IL-5 alone or in combination with SCF do not downregulate GILZ production by MCs.

Conclusions: These results show that GCs and IL-10 stimulate GILZ production by human MCs. As GILZ mediates anti-inflammatory effects of GCs in immune cells, we speculate that GILZ could account for the deactivation of MCs by GCs and IL-10.

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Glucocorticoids (GCs) are potent immunosuppressive agents which have pleiotropic actions on haematopoietic cells (1). Due to these properties, they are widely used in the treatment of chronic inflammatory diseases such as allergy and asthma (1). Among all effector cells in allergy and asthma, mast cells (MCs) are one target of GCs. The actions of GCs on MCs are not limited to an inhibition of their recruitment and survival in tissues (2). They also prevent the activation of resident MCs via their high-affinity IgE receptor (2, 3).

Most of the anti-inflammatory effects of GCs are mediated by the modulation of gene expression through either a direct interaction of the GC receptor to DNA (1) or an interference with the function of transcription factors such as NF- κ B and AP-1 (1).

Glucocorticoid-induced leucine zipper (GILZ) is one of the GC-induced genes, identified by comparing cDNAs from untreated and dexamethasone (DXM)-treated murine T lymphocytes (4). In humans, GILZ is expressed by lymphohaemopoietic cells (5). The over-

expression of its gene in DXM-treated or genetically modified immune cells prevents their activation by antigens (Ag) or pathogens according to the cell type (6–8). GILZ reduced the expression of the CD80 and CD86 co-stimulatory molecules on macrophages and their production of inflammatory chemokines (6). We recently demonstrated that GILZ downregulates the expression of CD80 and CD86 by dendritic cells (DCs) while upregulating the expression of tolerance-inducing molecules such as B7-H1 (Programmed death-ligand 1) and immunoglobulin-like transcript 3 (ILT3) (7). Besides modulating the phenotype of DCs, GILZ also affects their functions of Ag-presenting cells (7). In view of the importance of GILZ in mediating the immune suppressive effects of GCs, we investigated whether GILZ was produced by human MCs and whether GILZ gene expression was stimulated by GCs. We also studied the regulation of GILZ production under cytokine (IL-10, IL-4 and IL-5) or histamine stimulation.

Materials and methods

Cells and cultures

HMC-1 5C6 cell line was grown in Roswell Park Memorial Institute (RPMI) media 1640-Glutamax (Invitrogen, Cergy-Pontoise, France) supplemented with 10% foetal calf serum (FCS; Invitrogen), 100 IU/ml penicillin and 50 µg/ml streptomycin (Invitrogen).

The human MC line LAD-2, a kind gift of Dr AS Kirshenbaum (NIAID, Bethesda, MD) was grown in Iscove's modified Dulbecco's medium (IMDM) supplemented as previously described (9).

Highly purified CB-MCs were obtained by long term culture of cord blood progenitor cells as previously described (10).

For *in vitro* experiments, cells were seeded at 10^6 cells/ml and stimulated in their respective medium with various stimulants: recombinant human interleukin (rhIL)-4 (R&D Systems, Abingdon, UK), rhIL-5 (AbCys S.A., Paris, France), each used at a concentration of 20 ng/ml, or rhIL-10 from K. Moore (DNAX, Palo Alto, CA) used at a concentration of 20 ng/ml and 100 ng/ml. Histamine (Sigma-Aldrich, St-Quentin-Fallavier, France) was used at a concentration range of 10^{-4} to 10^{-6} M. DXM (Sigma-Aldrich) was used at a concentration range of 10^{-7} to 10^{-9} M.

Reverse transcription (RT)-PCR analysis

Total RNA was extracted using Trizol (Invitrogen). PCR reactions were performed as previously described (6).

Western blot analysis

Extracted proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, Western blotted and visualized using enhanced chemoluminescence (Amersham, Orsay, France) as previously described (6). Polyclonal antibodies directed against GILZ were kindly provided by M. Pallardy (INSERM UMR-S749, Châtenay-Malabry, France). Polyclonal anti-Akt antibody was purchased from Euromedex (Mundolsheim, France) and served as control load. The secondary antibody was a horseradish peroxidase-conjugated anti-rabbit (Tebu-Bio SA, Le Perray-en-Yvelines, France).

Immunocytochemistry

Cytoentrifugation slides were prepared with 10^4 LAD-2 MCs per glass slide. After incubation first with a goat anti-human polyclonal antibody against GILZ (Santa-Cruz Biotechnology, Heidelberg, Germany) and second with biotinylated anti-goat immunoglobulins (Vector, Burlingame, CA), immunocytochemical staining was conducted with alkaline phosphatase-conjugated streptavidin (Biogenex, San Ramon, CA). Staining was completed after incubation with substrate-chromogen Fast Red (Sigma-Aldrich).

Results

Human MCs are a source of GILZ

We looked for the expression of GILZ mRNA and protein by human MCs cultured under normal conditions. The production of GILZ was evaluated by RT-PCR and Western blotting in HMC-1 5C6 MC line and CB-MCs. GILZ mRNA was detected in unstimulated

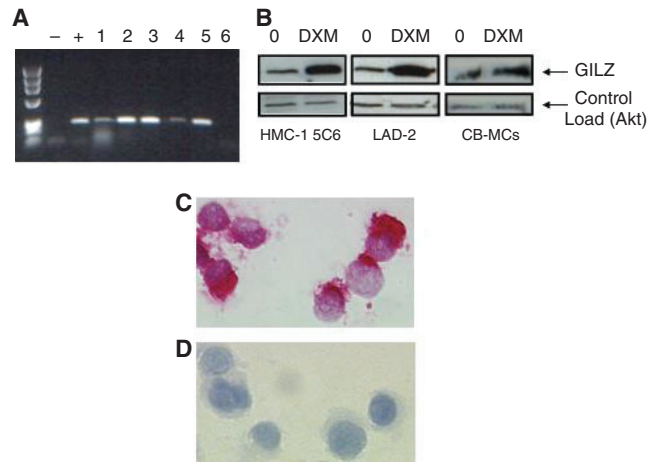


Figure 1. GC-stimulated GILZ gene expression in human MCs. Production of GILZ by human MCs cultured alone or with DXM was studied by using RT-PCR (A), Western blotting (B) or immunocytochemistry (C, D). (A) GILZ RT-PCR in HMC-1 5C6 MCs (cDNA diluted at 1 : 10 [1], 1 : 100 [2] and 1 : 1000 [3]) and in CB-MCs (cDNA diluted at 1 : 10 [4], 1 : 100 [5] and 1 : 1000 [6]). (B) Production of GILZ by HMC-1 5C6 MC line, LAD-2 MC line and CB-MCs cultured for 4 h alone (lane 0) or with an optimal concentration of DXM (10^{-7} M) was studied by using Western blotting. (C, D) Expression of GILZ gene was analysed by immunohistochemistry on cytoentrifugation slides of DXM-treated LAD-2 MCs. Staining with GILZ antibody (C) and negative control (D), omission of the primary antibody). Slides were counterstained with Mayer's haematoxylin solution. Results presented are those of one of three representative experiments.

HMC-1 5C6 MCs and CB-MCs (Fig. 1A). The results obtained were consistent with Western blot studies, showing expression of GILZ in unstimulated HMC-1 5C6 MC line and CB-MCs (Fig. 1B). GILZ protein was also detected in unstimulated LAD-2 MC line by Western blotting (Fig. 1B). Studies of GILZ gene expression by immunocytochemistry revealed a predominant cytoplasmic localization of GILZ in MCs (Fig. 1C,D).

GCs stimulate GILZ synthesis by human MCs

We performed *in vitro* experiments to study the regulation of GILZ by GCs. HMC-1 5C6 cells, LAD-2 MCs and CB-MCs were cultured alone or in the presence of DXM, and GILZ gene expression was assessed by Western blotting. The production of GILZ was greatly enhanced in the two MC lines and in CB-MCs treated 4 h with DXM (Fig. 1B).

The kinetics of GILZ production and dose effects of DXM was determined using HMC-1 5C6 MCs. Irrespective of the incubation time, DXM was effective on GILZ production at 10^{-7} and 10^{-8} M but not at 10^{-9} M (Fig. 2A). Expression of GILZ was enhanced as early as 1 h following DXM addition. It peaked at 4 h and

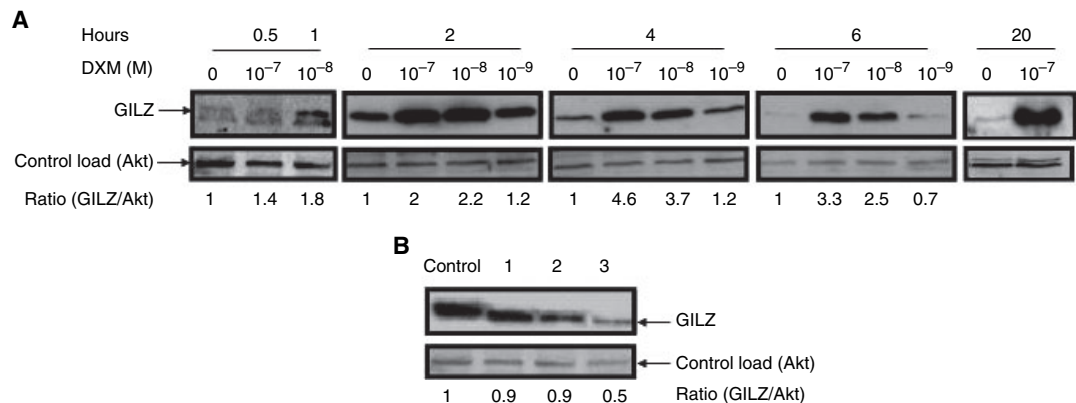


Figure 2. Kinetics of GILZ gene induction by GCs. (A) GILZ protein in Western blot studies with HMC-1 5C6 cells cultured for varying time intervals (from 30 min to 20 h) alone (0) or with DXM used at a range concentration from 10^{-7} to 10^{-9} M. (B) Turnover of DXM-induced GILZ protein. GILZ gene expression was enhanced by stimulating HMC-1 5C6 MCs with DXM (10^{-7} M) for 2 h (lane control). Then, cells were washed and tested for the levels of GILZ protein after 2 h (lane 1), 4 h (lane 2) and 6 h (lane 3). GILZ expression was quantified by densitometric analysis and normalized to the densitometric values of Akt (ratio GILZ/Akt). Results presented are those of one of three representative experiments.

remained elevated after a long period of stimulation (Fig. 2A). We then evaluated the time necessary for MCs to return to basal level of GILZ after DXM treatment was stopped (Fig. 2B). HMC-1 5C6 MCs were treated with DXM 2 h which enhanced GILZ gene expression by 50%, followed by a washing step. Cells were then cultured in DXM-free medium and tested for the production of GILZ at different time course. The level of GILZ was stable until 4 h after the end of DXM treatment and returned to basal conditions after 6 h (Fig. 2B).

IL-10 stimulates GILZ synthesis by human MCs

We investigated whether IL-10, similar to GCs, stimulated GILZ production. HMC-1 5C6 MCs and LAD-2 MCs were cultured alone or in the presence of IL-10, and GILZ gene expression was evaluated by Western blotting. Production of GILZ was upregulated in MCs cultured for 4 h with IL-10 (Fig. 3A). The effect of IL-10 was more pronounced on GILZ gene expression 20 h after its addition to MC cultures (Fig. 3A). The upregulation of GILZ production by IL-10 was also observed in LAD-2 MCs but was not as intense as observed with DXM (Fig. 3B).

Histamine, IL-4 and IL-5 do not regulate GILZ synthesis by human MCs

We investigated whether GILZ production could be modulated by histamine. HMC-1 5C6 MCs were cultured alone or in the presence of increasing concentrations of histamine from 10^{-6} to 10^{-4} M. Western blot analysis indicated that histamine did not affect the level of GILZ gene expression even at the highest concentration of 10^{-4} M (Fig. 4A) and after 20 h of incubation. We then

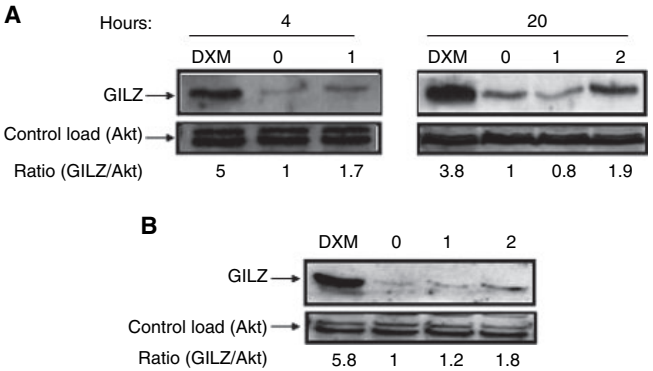


Figure 3. IL-10 induces GILZ gene expression in human MCs. Analysis of GILZ expression by Western blotting in HMC-1 5C6 (A) and LAD-2 (B) MCs cultured for 4 or 20 h alone (0) or with DXM (10^{-7} M, positive control) or IL-10 at a concentration of 20 ng/ml (lane 1) or 100 ng/ml (lane 2). GILZ expression was quantified by densitometric analysis and normalized to the densitometric values of Akt (ratio GILZ/Akt). Results presented are those of one of three representative experiments.

examined whether cytokines known to stimulate MCs when concomitantly present in their environment with SCF were able to modify GILZ production. IL-4 and IL-5 tested alone or in the presence of SCF did not influence the level of GILZ production in HMC-1 5C6 MCs (Fig. 4B,C).

Discussion

GILZ is produced by human T lymphocytes, macrophages and DCs, and its production is increased by GCs (6–8). In this work, we demonstrate that human MC lines and MCs derived from CD34⁺ progenitor cells also constitutively produce GILZ. We also show that GCs

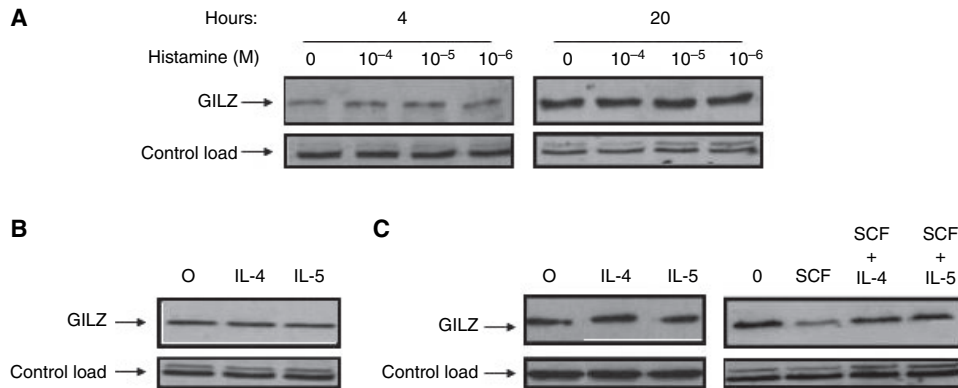


Figure 4. Histamine and Th2 cytokines do not downregulate GILZ production in human MCs. (A) GILZ protein in Western blot studies with HMC-1 5C6 MCs cultured alone (0) or with increasing concentrations of histamine for 4 or 20 h. (B, C) Production of GILZ evaluated after stimulation of HMC-1 5C6 cells for 4 h (B) or 20 h (C) with IL-4 (20 ng/ml) or IL-5 (20 ng/ml) alone or in combination with SCF (80 ng/ml). Results presented are those of one of three representative experiments.

stimulate *in vitro* GILZ synthesis by human MCs. Activation of GILZ gene expression by GCs is a rapid phenomenon, as DXM-treated MCs upregulate GILZ production as soon as 1 h after GC exposure. The level of GILZ production remains high as long as the treatment is maintained, but it decreases very quickly to its basal level when DXM exposure is stopped. The rapid modulation of GILZ gene expression is one of the important conditions to validate the concept that GILZ production could correspond in a state of activation/non activation of the cells.

Interleukin-10 also stimulates GILZ production by human MCs, although to a lesser extent than GCs. This result is particularly interesting given the large number of anti-inflammatory and immunosuppressive properties common to IL-10 and GCs. For example, both IL-10 and GCs are able to inhibit IgE activation of human MCs (11). The induction of GILZ production may be a common pathway used by GCs and IL-10 to deactivate MCs and prevent their activation by allergens. Interestingly, IL-10 is a major product of T-regulatory cells (12) and is believed to play a critical role in regulating allergic response (12, 13).

Histamine, a key mediator in allergy, also acts as a proinflammatory factor that is able to activate MCs (14). Our present results indicate that histamine does not downregulate GILZ gene expression. The Th2 cytokines, IL-4 and IL-5, which favour MC survival and/or proliferation with SCF (15) also failed to decrease the basal level of GILZ production in MCs.

Overall, our findings show that GCs and IL-10 stimulate GILZ production by human MCs. As GILZ appears to be a critical actor in the anti-inflammatory and immunosuppressive effects of GCs and IL-10 in both human macrophages and DCs (6, 7), we speculate that in MCs GILZ expression may also affect the balance between exacerbated activation and inactivation of these cells. Further studies will aim at identifying how GILZ affects MC activation.

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