Glucocorticoid-mediated inhibition of RANTES expression in human T lymphocytes

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Abstract The chemokine RANTES has been implicated in the pathogenesis of allergic inflammatory diseases including asthma and rhinitis which are frequently treated with glucocorticoids. We observed that dexamethasone dramatically inhibited RANTES mRNA expression dose dependently in anti-CD3 activated Hut-78 T cells and human PBMCs. Inhibition of RANTES expression did not appear to be secondary to IL-2 inhibition and required binding to the intracellular glucocorticoid receptor. The down-regulation of RANTES expression by glucocorticoids in T cells may directly contribute to the efficacy of these agents in suppressing cellular infiltration and to their anti-inflammatory properties.

Key words: RANTES; T cell; Glucocorticoid; Dexamethasone

1. Introduction

The pathogenesis of allergy and asthma is clearly associated with the release of proinflammatory mediators from eosinophils and other inflammatory cells recruited to the site of allergic inflammation. Although tissue infiltration by large numbers of eosinophils is well recognized, the processes that control eosinophil recruitment are not fully understood. Recently, the cytokine RANTES (regulated upon activation, normal T cell expressed and secreted), a member of the C-C family of chemokines, has been implicated as a pivotal mediator of eosinophil recruitment and subsequent inflammation [1]. The RANTES gene encodes an 8-kDa cytokine with potent chemotactic activity for eosinophils, basophils, monocytes, NK cells, and $CD45RO^+$ 'memory' $CD4^+$ T cells [2,3]. Diverse proinflammatory actions of RANTES include activation of eosinophils and T cells [4,5], adhesion of T cells to activated endothelium [6], and release of histamine from basophils [7]. Since RANTES is present at high levels in association with eosinophil-rich infiltrate in nasal polyps [8] and the bronchial epithelium of mild asthmatics [9], both clinical and biochemical data support a potentially critical role for RANTES in allergic inflammation.

Multiple cell types including macrophages, mast cells, fibroblasts, and tubular epithelial cells appear to contribute to an initial and transient production of RANTES at the sites of allergic inflammation [6,10]. T lymphocytes are then recruited to the area where they become activated by their cognate antigen and subsequently produce large and sustained levels of RANTES thereby amplifying and maintaining the inflammatory response. Thus, understanding the mechanisms that regulate RANTES expression in T lymphocytes may provide valuable insights for the therapeutic manipulation of allergic inflammation.

Anti-inflammatory therapy with glucocorticoids is of critical importance in the treatment of asthma. Although glucocorticoids are recognized to have anti-inflammatory effects including inhibition of cytokine and lymphokine expression (reviewed in [11]), reduction of T cell proliferation [12], and inhibition of eosinophil infiltration [13], the molecular basis for these pluripotent effects are not well characterized. In this study, we examined the effect of glucocorticoid treatment on RANTES expression in T cells and observed that expression in both anti-CD3 activated and resting T cells is dramatically inhibited by dexamethasone.

2. Materials and methods

2.1. Cell culture and reagents

The Hut-78 cell line was obtained through the American Type Tissue Collection (ATCC TIB 161), Rockville, MD. Cells were cultured in RPMI supplemented with 10% FCS (Sigma, St. Louis, MO), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 15 mM HEPES. PBMCs and Hut-78 cells were typically maintained at $4–6\times10^5$ cells/ml and were activated using immobilized OKT3 (anti-CD3) monoclonal antibody (ATCC, Rockville, MD) at 30 μg/100 mm tissue culture plate per 3×10^6 cells. Cells were also treated with varying concentrations of Dex (1–100 nM) (Sigma, St. Louis, MO), IL-2 (100 U/ml) (Biological Response Modifiers Group, NCI, Frederick, MD), and RU 40555 (1 μM) (Roussell Uclaf, Romainville, France).

2.2. Isolation of PBMCs

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient separation using Histopaque according to the manufacturer's protocol (Sigma, St. Louis, MO) from whole blood donated from volunteers (American Red Cross Blood Bank, Boise, ID). Lymphocytes were depleted of monocytes by plastic adherence as previously described [14]. Purity of lymphocyte populations was determined by Wright Giemsa staining and preparations typically contained >85% lymphocytes. Viability was >98% as assessed by trypan blue dye exclusion.

2.3. RNA isolation and Northern blot analysis

Total cellular RNA was isolated from cell samples (3×10^6) using the guanidinium isothiocyanate (GITC)/cesium chloride method [15]. Briefly, cells were solubilized in 4 M GITC and overlaid on a 5.7 M CsCl cushion. Samples were ultracentrifuged at $150\,000\times g$ for 17 h. The RNA pellet was resuspended, ethanol precipitated and quantitated. RNA (20 μg) was denatured in 4×sample buffer and electrophoresed in a 1.25% agarose-formaldehyde gel [15]. The gel was transferred to a nylon membrane and the nucleic acids immobilized by UV cross-linking. The filters were prehybridized at 42°C for 4 h in 50% formamide, 5×SSC, 50 mM potassium phosphate pH 8.0, 5×Denhardts' solution and 100 μg/ml denatured salmon sperm DNA. Hybridizations were performed at 42°C for 18 h in the above solution substituting 20 mM potassium phosphate pH 6.5 and 1×Denhardts'

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Abbreviations: PBMC, peripheral blood mononuclear cells; DEX, dexamethasone.

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solution, and adding 2×10^6 cpm of radiolabeled probe per ml of solution. Filters were sequentially hybridized with a ^{32}P-labeled 410 bp *Eco*RI/*Apa*I RANTES probe (ATCC) and the human GAPDH cDNA (ATCC). Blots were washed in 0.1×SSC and 0.1% SDS at 60°C and exposed to X-ray film. Equivalent loading of RNA was determined by ethidium bromide staining of ribosomal RNA and by detection of GAPDH transcripts on Northern blots.

3. Results

3.1. T cell Ag receptor engagement rapidly induces RANTES expression in T cells

Antigen-dependent T cell activation is induced by the interaction of the CD3 component of the T cell receptor with an appropriately presented antigen [16]. CD3 receptor signaling can also be initiated using immobilized anti-CD3 (OKT3) antibodies. We examined whether CD3-mediated signaling alters the expression of RANTES using the Hut-78 T cell line as an initial model system. This CD4$^+$ cell line was derived from a cutaneous T cell lymphoma and expresses a 'mature' T cell phenotype demonstrated by constitutive expression of IL-2 and IL-2 receptors [17]. We observed that the activation of Hut-78 cells with immobilized anti-CD3 antibody results in a rapid induction of RANTES mRNA (Fig. 1A) with a 7-fold increase over constitutive expression. In contrast to the late transcriptional increases previously described using peripheral T cells [18], dramatically elevated levels of RANTES message were observed as early as 3 h post receptor ligation.

Although the Hut-78 cell line constitutively expresses moderate levels of both IL-2 and IL-2 receptor, this cell line is responsive to exogenous IL-2 both in terms of cytokine production and proliferation [17]. To determine whether increased IL-2 production caused by CD3 receptor ligation [19] is responsible for up-regulation of RANTES expression, unactivated cells were treated for 24 h with 100 U/ml IL-2. As shown in Fig. 1B, IL-2 treatment fails to upregulate RANTES expression demonstrating the involvement of distinct CD3 receptor mediated pathways in RANTES up-regulation. The ineffectiveness of IL-2 in altering the expression of RANTES was also observed in human PBMCs (data not shown).

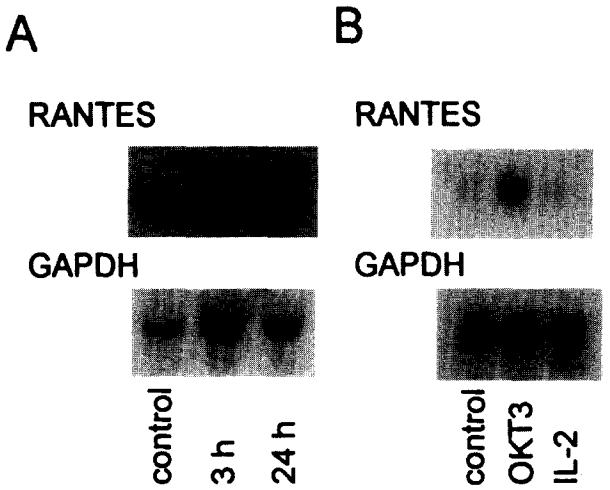

Fig. 1. Time course of RANTES expression in anti-CD3 activated Hut-78 cells. (A) Hut-78 cells were stimulated with immobilized OKT3 antibody for 0, 3, or 24 h and the level of RANTES mRNA determined by Northern analysis. (B) Cells were cultured for 24 h in either complete media (lane 1), in the presence of immobilized OKT3 antibody (lane 2), or with 100 U/ml of IL-2 (lane 3) as indicated and RNA expression analyzed by Northern blots. Equivalent RNA loading was verified by re-probing with GAPDH and monitoring rRNA fluorescence intensity (not shown). Representative autoradiograms are presented.

3.2. Dexamethasone inhibits constitutive and anti-CD3 inducible RANTES expression

Because glucocorticoids are commonly used in the treatment of allergic diseases in which RANTES may be of pathologic significance, the effect of DEX on RANTES expression in unactivated and activated T lymphocytes was examined. As shown in Fig. 2A, 24 h treatment with 100 nM DEX dramatically decreased RANTES expression in unactivated Hut-78 cells. Expression of RANTES in Hut-78 cells stimulated with anti-CD3 antibody for 24 h was also inhibited by 100 nM DEX (Fig. 2B). Dexamethasone had no effect on either constitutive or anti-CD3 stimulated RANTES expression when

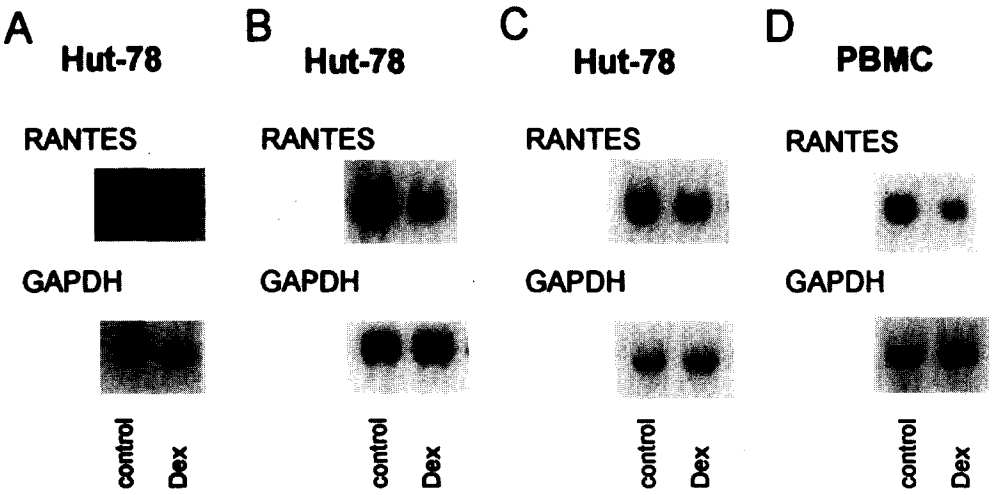

Fig. 2. Dexamethasone inhibits the expression of RANTES in human T cells. Unactivated Hut-78 cells (A), 24 h anti-CD3 activated Hut-78 cells (B), 3 h anti-CD3 activated Hut-78 cells (C), and 40 h anti-CD3 plus IL-2 treated human PBMCs (D) were concurrently treated with dexamethasone (100 nM) or vehicle for various time as indicated and RANTES expression determined by Northern analysis. Equivalent RNA loading was verified by re-probing with GAPDH and representative autoradiograms are presented.

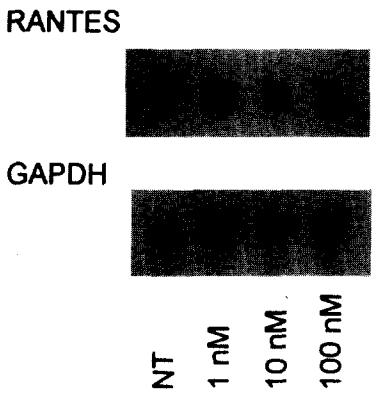

Fig. 3. Dose response of Dex mediated inhibition of RANTES expression. Hut-78 cells were activated with anti-CD3 antibody and concurrently treated with increasing concentrations of Dex as indicated. After 24 h of culture, RANTES mRNA expression was determined by Northern analysis. Equivalent RNA loading was verified by re-probing with GAPDH and representative autoradiograms are presented.

dexamethasone and anti-CD3 exposure was limited to 3 h (Fig. 2C). These slow kinetics of action suggest that the inhibitory properties of DEX on RANTES expression require de novo protein synthesis.

To determine whether DEX also inhibits RANTES expression in primary lymphocytes, human PBMCs were activated with anti-CD3 plus IL-2 and cultured in the presence or absence of 100 nM DEX for 40 h. Viability in DEX-treated cells was assessed via trypan blue dye exclusion to be $>90\%$. We observed a dramatic and long-lasting down-regulation of RANTES expression clearly visible in 40 h treated cells (Fig. 2D). Because DEX can decrease IL-2 production, experiments were performed to determine whether decreased RANTES expression was secondary to the effects of decreased IL-2. We observed that DEX inhibited RANTES expression in anti-CD3 activated PBMCs in either the presence (Fig. 2) or absence of exogenous IL-2 (unpublished data).

3.3. Inhibition of RANTES expression by DEX is concentration-dependent and requires binding to the GC receptor

To determine the minimum concentration of DEX required to inhibit RANTES expression, various amounts of DEX were added to Hut-78 cells for 24 h at the time of T cell activation. As shown in Fig. 3, dose-dependent inhibition of RANTES expression was observed with diminished RANTES expression observable with concentrations as low as 1 nM.

To determine whether the inhibitory activity of DEX on RANTES expression requires binding to its intracellular receptor, we evaluated the effects of the steroid hormone antagonist RU40555. Incubation of Hut-78 cells with RU40555 did not significantly alter the expression of RANTES (Fig. 4). However, RU40555 dramatically abrogated the inhibitory effects of 100 nM DEX indicating a mechanism requiring the formation of a functional DEX-GC receptor complex.

4. Discussion

These studies provide the first demonstration that expression of the RANTES gene in human T cells is sensitive to inhibition by glucocorticoids. We observed that DEX treatment dose-dependently suppresses RANTES mRNA expression in both unactivated and antigen-receptor activated PBMCs and Hut-78 cells. This dramatic inhibition was found to result typically in a 66% and 64% decrease relative to the level of expression observed in activated control Hut-78 cells and PBMCs, respectively. Decreased RANTES was observed with concentrations as low as 1 nM which is well within in vivo concentrations expected following systemic glucocorticoid administration. Given the pivotal role of RANTES in allergic conditions associated with eosinophil infiltration, the inhibitory effects of DEX on RANTES expression may be intimately linked to the anti-inflammatory properties of glucocorticoids and to their clinical effectiveness in controlling airway inflammation.

At present, the specific biochemical and molecular mechanisms of RANTES inhibition by DEX remain unclear. However, our results indicate that mechanisms independent of decreased IL-2 production caused by DEX treatment are responsible for suppression of RANTES expression as exogenous IL-2 failed to alter the inhibitory effects of DEX. It is plausible that glucocorticoid inhibition may be mediated through κB-like sites previously implicated in the control of RANTES transcription [20,21]. It has recently been shown that glucocorticoids activate IκB [22], an inhibitor of the κB-family of transcription factors, and may prevent the κB-family of transcription factors from up-regulating RANTES expression. These potential mechanisms of glucocorticoid action remain an important area for future work.

We have observed that RANTES mRNA is markedly and rapidly induced (3 h) following anti-CD3 activation (Fig. 1A) in Hut-78 cells compared to the late up-regulation (days 3–7) initially reported for primary T cells following antigen receptor signaling. Immediate-early induction kinetics of RANTES has also been observed in other cell types including macrophages, fibroblasts, and epithelial cells [6,10]. Recent data suggest the immediate-early induction of RANTES observed in macrophages and other non-hematopoietic cells may be

Fig. 4. The glucocorticoid receptor antagonist RU40555 prevents inhibition of RANTES expression by Dex. Hut-78 cells were activated with anti-CD3 antibody and concurrently treated with Dex (100 nM) and/or RU40555 (1 μM). After 24 h of culture, RANTES mRNA expression was determined by Northern analysis. Equivalent RNA loading was verified by re-probing with GAPDH and representative autoradiograms are presented.

mediated through the Rel heterodimers p50-p65 transcription factors [21]. These factors have been shown to be transiently induced early in T cell activation [23] and may also be involved in the immediate-early induction of RANTES expression in activated Hut-78 cells. Interestingly, it appears that these factors do not function analogously during the initial stages of primary T cell activation [21] as demonstrated by the characteristic absence of early RANTES expression suggesting the involvement of distinct elements in the delayed up-regulation of primary T cells. Given the importance of RANTES in the pathogenesis of both allergic inflammation and AIDS [24], the comparison and identification of the DNA-binding factors causing the early up-regulation of RANTES in Hut-78 T cells compared to the very late up-regulation in primary T cells following antigen receptor ligation should provide important information for understanding the control of RANTES expression and potentially impact the therapy of a variety of disease states. The mechanisms of RANTES up-regulation and glucocorticoid inhibition may be of main importance and are clearly relevant in the very common therapeutic use of glucocorticoids.

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