Human Mast Cells Modulate Proliferation and Cytokine Production by CD8+ T Lymphocytes

F.L.	Francina L.	de Pater-Huijsen ^{a,b}
M.J.	Mariëlle J.	de Riemer ^{a,b}
R.M.R.	Richard M.R.	Reijneke ^{b,c}
M.	Marjolein	Pompen ^{a,b}
R.	René	Lutter ^{a,b}
H.M.	Henk M.	Jansen ^a
T.A.	Theo A.	Out ^{b,c}

^aDepartment of Pulmonology, ^bClinical and Laboratory Immunology Unit, Academic Medical Center and ^cLaboratory for Experimental and Clinical Immunology (CLB), Amsterdam, The Netherlands

Key Words

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Correspondence to: Dr. T. A. Out, PhD, Clinical Immunology Laboratory, Academic Medical Center B1-236, PO Box 22660, NL-1100 DD Amsterdam (The Netherlands), Tel. (31) 20 5662893/5662528, Fax (31) 20 6917584, E-Mail t.a.out@amc.uva.nl

Introduction

In patients with allergic asthma, both mast cells and T lymphocytes are believed to be involved in the airway pathophysiological processes. Cross-linking of mast cell IgE receptors by allergen binding leads to subsequent release of mediators. In addition to the immediate effects on broncho-constriction, these mediators also participate in inflammatory reactions. Activation of T cells was found to be correlated with disease severity [1]. CD4+ T cells from bronchoalveolar lavage fluid and bronchial biopsies from subjects with allergic asthma showed increased Th2 activity as compared to control subjects [2, 3]. The Th2-derived cytokines IL-4, IL-5 and IL-13 may play a pivotal role in the pathophysiology of allergic asthma in that they stimulate B cell IgE production and eosinophil functions. CD8+ T lymphocytes may exert suppressive regulatory functions by the production of substantial amounts of IFN- γ , resulting in the inhibition of IgE production [4]. How T cell reactions are regulated in vivo is unclear. Probably, antigen-presenting cells play an important role [5]. On the other hand, mast cells produce a large number of immunomodulatory cytokines and may have important immunoregulatory functions in allergic inflammation. We show here that the human mast cell line HMC-1 influences properties of CD8+ T cells.

Materials and Methods

Human mast cell line HMC-1 was obtained from a patient with mast cell leukemia (kindly donated by Dr. J.H. Butterfield, Mayo Clinic, Minneapolis, Minn., USA). When indicated, 0.4×10^6 /ml cells were stimulated by phorbol myristate acetate (Sigma, St. Louis, Mo., USA), final concentration 10 ng/ml, and calcium ionophore A23187 (Sigma), final concentration 10 μ g/ml. All cells were incubated for 30 min at 37°C, after which the cells were pelleted and resuspended in fresh medium. The cells were irradiated with 3,000 rad of γ -irradiation at 0°C. Next, the cells were pelleted once more and resuspended at 0.4×10^6 ml in Iscove's Modified Dulbecco's Medium (medium I; Gibco BRL, Paisley, UK) containing 10% human pool serum (Biowhittaker, Walkersville, Md., USA), 2×10^{-5} M β -mercaptoethanol (Merck, Munich, Germany), 2 mM sodium pyruvate (Merck), penicillin (100 U/ml; Gist Brocades, Delft, The Netherlands) and streptomycin (100 μ g/ml, Gibco).

Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Isopaque ($d = 1.078$) centrifugation of a buffy coat from a healthy subject. T cell clones were prepared as described [6]. At weekly intervals, the clonal cells were stimulated with phytohemagglutinin, IL-2 and irradiated PBMC (3,000 rad). Three days before performing the assay, the medium was removed and the cells were suspended in medium I. To assess cytokine production, 40,000 clonal cells were incubated in plates coated with anti-CD3 (16A9, ascites; gift from Dr. R. A.W. van Lier) with or without (stimulated) HMC-1 cells for 24 h, after which the supernatant was harvested. Supernatants of 5 wells were pooled and stored at -20°C. IFN- γ and IL-5 contents were measured by ELISA [6]. IL-4 levels were assessed using the PeliKine-com-pact IL-4 kit from CLB (Amsterdam, The Netherlands). Proliferation was assessed by measuring [3 H]-thymidine incorporation in parallel incubations [6]. Proliferation data are shown as the mean $\pm$ SD of quintuplicates.

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Results and Discussion

Stimulation of HMC-1 cells with PMA and A23187 resulted in increased production of several mediators and cytokines, e.g. IL-8 (data not shown). [3 H]-thymidine incorporation of a CD8 $^+$ T cell clone (1B7) stimulated by anti-CD3 alone was $9,131 \pm 1,076$ cpm. Addition of resting HMC-1 cells increased incorporation to $14,612 \pm 597$ cpm. However, stimulated HMC-1 cells caused a decrease in [3 H]-thymidine incorporation to $4,258 \pm 855$ cpm. To study the effects of non-specific cell-cell interactions, we incubated the clonal cells with the same number of irradiated non-stimulated PBMC instead of mast cells. Incorporation was now $13,157 \pm 1,898$ cpm, indicating specific inhibition by stimulated HMC-1 cells.

The effects on cytokine production of CD8 $^+$ T cells are depicted in figure 1. Upon anti-CD3 stimulation (= control), these clonal cells produced detectable levels of all three cytokines tested. Resting mast cells increased IFN- γ and IL-5 production to 273 and 140% of the control, respectively, whereas IL-4 production was decreased to 43% of the control. Stimulated mast cells increased IFN- γ production to 819% as compared to anti-CD3 alone. IL-4 production was only slightly raised (114% of the control), whereas IL-5 production was decreased to 39% of the control. PBMC increased IFN- γ and IL-5 production to the same extent as the resting HMC-1 mast cells did. However, IL-4 production was increased by PBMC.

These results show that HMC-1 cells are able to modulate proliferative and cytokine production responses of a CD8+ T cell clone in vitro. Activated mast cells seem to

Fig. 1. IFN- γ , IL-4 and IL-5 production by CD8+ T cell clone 1B7. Cells were seeded at 40,000 cells/well and stimulated with coated anti-CD3. Supernatants were harvested after 24 h. +HMC = Irradiated resting HMC-1 mast cells; +HMCstim = irradiated PMA plus A23187-stimulated HMC-1 cells; +PBMC = irradiated PBMC.

drive this CD8+ T cell clone towards a more pronounced Th1-type of response, simultaneously decreasing T cell numbers. This might be a negative feedback mechanism operating in allergic subjects, by which Th2-driven IgE production and eosinophilia are counteracted.

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de Pater-Huijsen/de Riemer/Reijneke/ Pompen/Lutter/Jansen/Out

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