Cytokine Production by Highly Purified Human CD8+ T Cells

Y.		Li
D.		Richards
A.		Noble
D.M.		Kemeny

Department of Allergy and Respiratory Medicine, Guy's and St Thomas's Hospital, St Thomas's Site, London, UK

Key Words

CD8
Tcell
Interleukin-4
Interferon- γ

Abstract

We have systematically investigated the capacity of highly purified human peripheral CD8+ T cells to produce interleukin (IL)-4 and interferon (IFN)- γ when triggered by different stimuli. CD8+ T cells were isolated from peripheral blood by positive selection to > 99% purity and stimulated with one of three different stimuli: phytohaemagglutinin (PHA) and IL-2, phorbol myristate acetate (PMA) and ionomycin, and plate-bound anti-CD3 and PMA. On their own, ionomycin and IL-2 failed to stimulate significant CD8+ T cell proliferation while PHA, plate-bound anti-CD3 and PMA induced weak proliferation. A combination of PHA and IL-2, PMA and ionomycin, or plate-bound anti-CD3 and PMA all induced vigorous CD8+ T cell proliferation. IFN- γ was produced following all three stimuli, but was greatest from cells cultured with PMA and ionomycin. However, IL-4 secretion was only detected in cell cultures stimulated with PMA and ionomycin. These results indicate that, with sufficient stimulation, human CD8+ T cells have the potential to produce Th2 as well as Th1 cytokines.

Correspondence to: Dr. D. M. Kemeny, MD, Department of Allergy and Respiratory Medicine, Guy's and St Thomas's Hospital, St Thomas's Site, London SE1 9RT (UK)

Introduction

Methods

CD4+ T cells can be subdivided according to the cytokines they secrete [1] but there have been very few systematic studies of CD8+ T cell cytokine secretion [2]. CD8+ T cells play an essential role in the clearance of virally infected cells, induction of immunity to parasite infection and regulation of IgE production [3,4]. CD8+ T cells are reported to be major interferon (IFN)- γ -producing cells and poor producers of interleukin (IL)-4 [5, 6]. The aim of this study was to systematically investigate IL-4 and IFN- γ -production from highly purified human CD8+ T cells using a number of different stimuli.

CD8+ T cells were isolated from peripheral blood to a purity of 99.9% and cultured at 5×10^5 cells/ml with various stimuli-phytohaemagglutinin (PHA) (1 μ g/ml), IL-2 (20 IU/ml), phorbol myristate acetate (PMA) (10 ng/ml), ionomycin (400 ng/ml), plate-bound anti-CD3 (50 ng/well) – at 37° C for 24 h pulsed with 0.5 μ Ci/well of [3H]-thymidine overnight, harvested and counted. Results are expressed as counts/min. Cytokine production from purified CD8+ T cells was determined following culture of 1 ml (1×10^6 cells/ml) in 24-well plates with various stimuli. Supernatants were collected 24 h later and cytokines measured by ELISA. The lower limit of detection of the assays was 30 pg/ml.

Table 1. Effects of different stimulæ on the proliferation and cyto-kine profile of freshly stimulated, highly purified, human CD8+ T cells

cant CD8+ T cell proliferation while PHA and plate-bound anti-CD3 and PMA induced very weak CD8+ T cell proliferation (table 1). A combination of PHA and IL-2, PMA and ionomycin, and plate-bound anti-CD3 and PMA all induced vigorous CD8+ T cell proliferation. IFN- γ production was induced by all three stimuli, but was greatest from cells cultured with PMA and ionomycin. In contrast, IL-4 secretion was only detected in cell cultures stimulated with PMA and ionomycin. These data show that a combination of PMA and ionomycin induced efficient proliferation of purified human CD8+ T cells, that optimal IFN- γ production from purified human CD8+ T cells was induced by this stimulus, and that IL-4 was only produced by purified CD8+ T cells when the cultures were stimulated with PMA and ionomycin.

Results and Conclusions

Acknowledgement

Purified CD8+ T cells were stimulated with PHA, IL-2, PMA, ionomycin or anti-CD3 alone or in combination. On their own, ionomycin and IL-2 failed to stimulate signifi-

This work was supported by a grant from Bayer-Yakuhin, Kobe, Japan.

References

- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T-cell clone. I: Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-2357.
- Sieling PA, Abrams JS, Yamamura M, Salgame P, Bloom BR, Rea TH, Modlin L: Immunosuppressive roles for IL-10 and IL-4 in human infection: In vitro modulation of T-cell response in leprosy. *J Immunol* 1993;150:5501-5510.
- Kemeny DM, Diaz-Sanchez D, Holmes BJ: CD8+ T cells in allergy. *Allergy* 1992;47:12-21.
- Kemeny DM, Noble A, Holmes BJ, Diaz-Sanchez D: Immune regulation: A new role for the CD8+ T cell. *Immunol Today* 1994; 15:107-110.
- Cardell S, Sander B, Moller G: Helper interleukins are produced by both CD4 and CD8 splenic T cells after mitogen stimulation. *Eur J Immunol* 1991;21:2495-2500.
- Paliard X, de Waal Malefijt R, Yssel H, Blanchard D, Chretien I, Abrams J, De Vries J, Spits H: Simultaneous production of IL-2, IL-4, and IFN- γ by activated human CD4+ and CD8+ T-cell clones. *J Immunol* 1988;141:849-855.