Cytokine Production by Highly Purified Human CD8+ T Cells

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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.

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 5×10⁵ cells/ml with various stimuli-phytohae-magglutinin (PHA) (1 µg/ml), IL-2 (20 IU/ml), phorbol myristate acetate (PMA) (10ng/ml), ionomycin (400ng/ml), plate-bond anti-CD3 (50 ng/well) – at 37° C for 24 h pulsed with 0.5 µCi/well of [3H]-thy-midine overnight, harvested and counted. Results are expressed as counts/min. Cytokine production from purified CD8+ T cells was determined following culture of 1 ml (1×10⁶ 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 stimuli on the proliferation and cytokine 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 significant proliferation. This work was supported by a grant from Bayer-Yakuhin, Kobe, Japan.

References