

The role of CD8⁺ T cells in immunoglobulin E regulation

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The link between immunoglobulin E (IgE) antibodies and environmental allergens, allergic sensitization and atopic diseases such as asthma, rhinitis and eczema is well established, yet treatment of these diseases is largely symptomatic and fails to correct the underlying immune disorder. B-cell production of IgE depends on interleukin 4 (IL-4) and is inhibited by interferon (IFN- γ). These cytokines are produced by T-helper 2 (Th2) and T-helper 1 (Th1) CD4⁺ T cells respectively. In atopic dermatitis skin and asthmatic lung, Th2 cells predominate but antigen-specific CD4⁺ T-cell clones derived from peripheral blood are a mixture of Th1, Th2, and Th0. Circulating allergen-specific CD4⁺ T cells differentiate into Th1- and Th2-like clones in the presence of IL-12 and IL-4 respectively. In rats, CD8⁺ T cells are potent regulators of IgE responses *in vivo*, although the mechanism for this is still unclear. CD8⁺ T-cell depletion in immunized animals prolonged the IgE response but prior depletion did not. Indeed, prior depletion of CD8⁺ T cells actually inhibited IgE production, suggesting that there may be more than one type of IgE regulatory CD8⁺ T cell. This was supported by the observation that CD8⁺ T cells inhibited the differentiation of both Th1 and Th2 CD4⁺ T cells *in vitro* as well as IL-4- but not IL-2-induced Th2 CD4⁺ T-cell growth. The existence of distinct CD8⁺ T-cell subsets is supported by the observation that IL-4 and IFN- γ regulate the growth and differentiation of CD8⁺ T cells. IL-4 inhibits the development of CD8⁺ T cells that produce IL-2 and IL-6 and enhances the generation of cells that make IL-4 and IL-5. A combination of IL-4 and anti-IFN- γ enhanced IFN- γ and IL-10 production. Studies are continuing to characterize different CD8⁺ T-cell subsets at a clonal level and to determine their role in IgE regulation.

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Existing treatments for allergic disease such as immunotherapy (11) and more recently interferon- α (IFN- α) (2) are only partially effective at modifying the allergic immune response. Year-long injections of ragweed pollen have been shown to suppress the seasonal rise in ragweed-specific immunoglobulin E (IgE) (31), indicating that IgE responses can, at least partially, be modified. In order to do so more effectively, we need a better understanding of the process of IgE regulation. IgE production is under the control of T cells (10, 21) or more specifically T-cell cytokines. *In vitro*, interleukin-4 (IL-4) induces B cells to produce IgE (3), and this is inhibited by interferon- γ (IFN- γ) (27). *In vivo*, treatment of mice with anti-IL-4 (8) suppresses IgE production. IL-4 knockout (18) mice, which cannot make IL-4, are also unable to make IgE, confirming the essential requirement for this cytokine for IgE synthesis.

Work carried out in our laboratory (13, 16, 17) and elsewhere (20, 23) indicates that CD8⁺ T cells are involved in the regulation of the IgE response. It now appears that there are distinct subsets of CD8⁺ T cells that produce different combinations of cytokines and may play an important part in determining the growth and differentiation of CD4⁺ T cells and the immunoglobulin isotype expressed by B cells. Since receiving the Pharmacia award for 1991 (12), our research programme has sought to understand the differentiation of CD8⁺ T-cell subsets and to define the role that these cells play in CD4⁺ T-cell differentiation and IgE regulation.

The development of the model

Our attention was first focused on the possible role of CD8⁺ T cells during studies of IgE regulation in

animals. The immunization protocol used is based on the observation that virtually all people exposed to castor bean dust make IgE antibodies to castor bean proteins, regardless of their genetic background (12, 14, 15, 29). Investigation of this phenomenon in rats showed that the substance responsible for this is the toxic lectin, ricin (30), which potentiates both antigen-specific and total serum IgE levels (5). The unique features of the ricin-enhanced IgE response are: (i) that IgE responses can be induced in low as well as high IgE responder strains of rat, (ii) that these IgE responses can be boosted by repeated injection of ricin and antigen and (iii) that the IgE response induced in this way is long-lived (6).

The mechanism for ricin enhancement of the IgE response appears to lie in the killing or inactivation of a subpopulation of ricin-sensitive CD8⁺ T cells that are activated within 24 h of injection of the rats with soluble antigen. These early-activated CD8⁺ T cells appear to be targeted by ricin because they bear an increased number of ricin-binding glycoproteins on their surface (7). When adoptively transferred to

naive, syngeneic recipients early-activated CD8⁺ T cells suppressed the IgE response by up to 95% (7). CD8⁺ T cells active later in the response have also been described in rats (20) and mice (23) following antigen inhalation.

In vivo CD8⁺ T-cell depletion

To further investigate the role of CD8⁺ T cells in IgE regulation, rats were depleted of CD8⁺ T cells *in vivo* using the anti-CD8⁺ monoclonal antibody OX-8 administered prior to and following immunization. High levels of IgE were sustained in immunized rats depleted of CD8⁺ T cells on day 33 (Fig. 1a). We therefore postulated that, if CD8⁺ T cells were removed prior to immunization, the rats would produce very large amounts of IgE when subsequently immunized. Surprisingly, CD8⁺ T-cell depletion prior to immunization did not enhance the IgE response but actually reduced it (Fig. 1b). Thus, it seems that some CD8⁺ T cells may actually contribute to the IgE response and that there may be

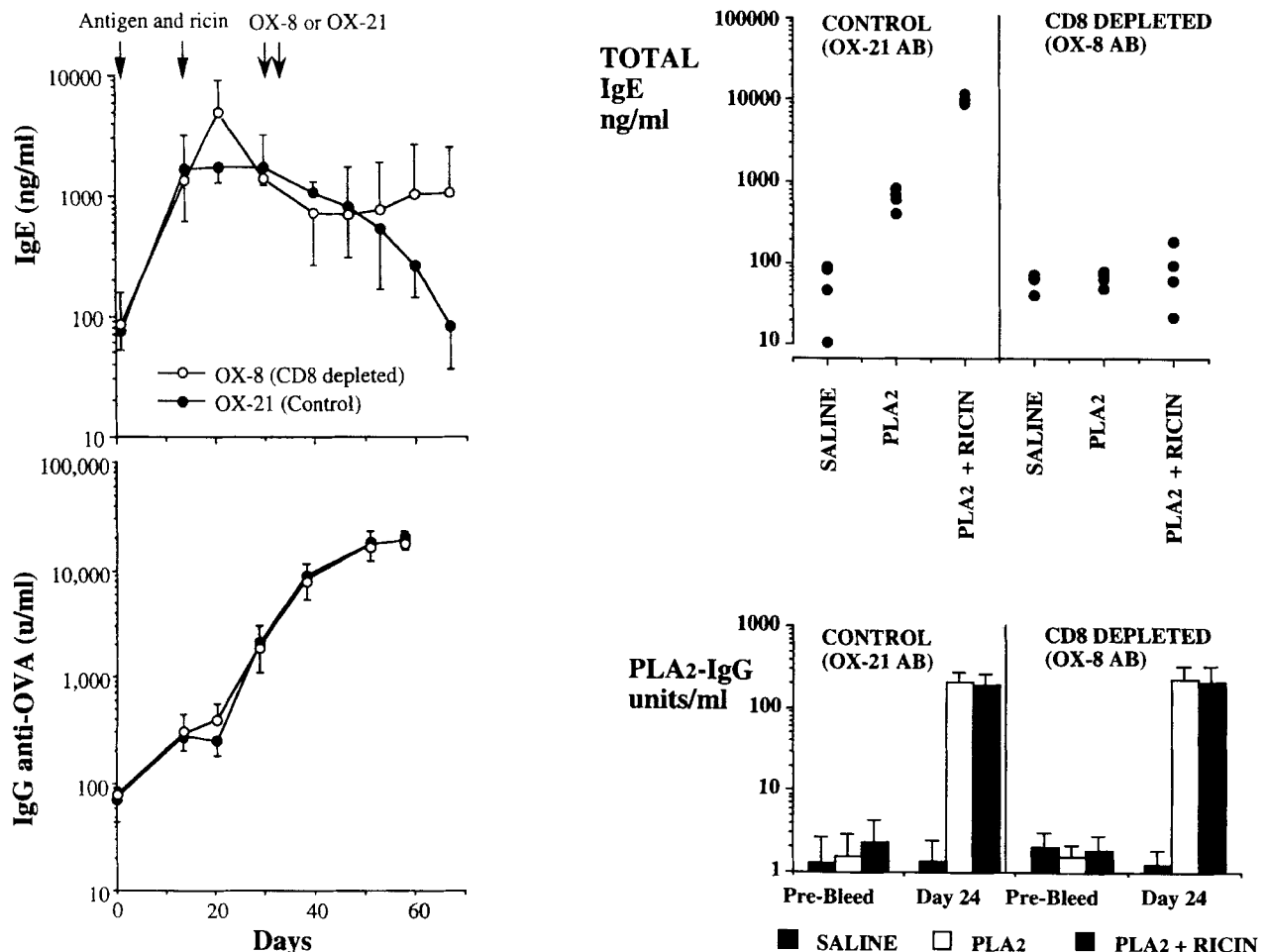


Fig. 1. The effect of CD8 depletion on (A) the established IgE response and (B) the primary IgE response.

more than one type of immunoregulatory CD8⁺ T cell. There are a number of different ways in which CD8⁺ T cells could regulate IgE. They could suppress B cell-IgE synthesis by secretion of IFN- γ or they might act indirectly by modulating the differentiation and function of the Th2-type CD4⁺ T cells. We have elected to investigate the latter possibility and have studied the effect of CD8⁺ T cells on CD4⁺ T-cell growth and differentiation. First we determined the effect of IL-4 and IFN- γ on rat CD4⁺ T cell growth and differentiation.

Growth and differentiation of Th1 and Th2 CD4⁺ T cells

The CD4⁺ T-cell culture system we developed is similar to that described by other investigators (9, 28) with certain modifications (Fig. 2). In most cases we were limited to assaying cytokine RNA message, as assays for secreted protein were only available for rat IFN- γ and IL-2. For studies of CD4⁺ T cell differentiation splenic lymphocytes were cultured for 4 days with concanavalin A (Con A) re-stimulated with phorbol-12-myristate-13-acetate (PMA) and ionomycin and RNA extracted for reverse transcription polymerase chain reaction (RT-PCR). In the absence of IL-4, cultured spleen cells lost the capacity to produce IL-4 and IL-5 (Fig. 3a). This is in contrast to the data obtained in mice, where addition of Con A alone or T cell receptor ligation appear to be a sufficient stimulus for the generation of Th2-like cells (24–26). It seems likely that this difference is at least partly due to endogenously secreted IFN- γ , as the capacity to produce IL-4 and IL-5 was restored if the cells were cultured in the presence of anti-IFN- γ . Addition of IL-4 and anti-IFN- γ permitted an expansion of cells with a Th2 cytokine profile, confirming the observations made in mice on the

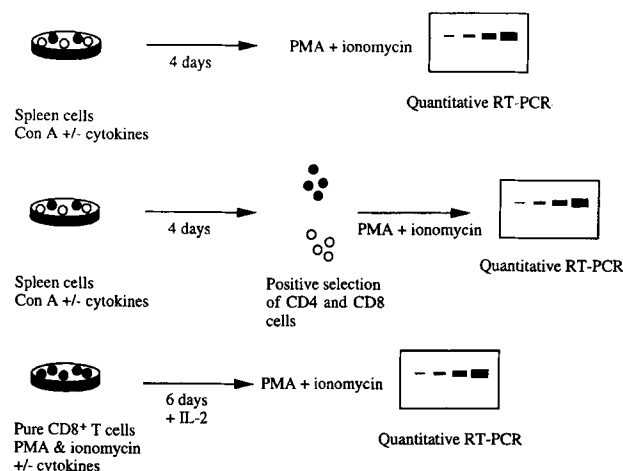


Fig. 2. T-cell differentiation assay.

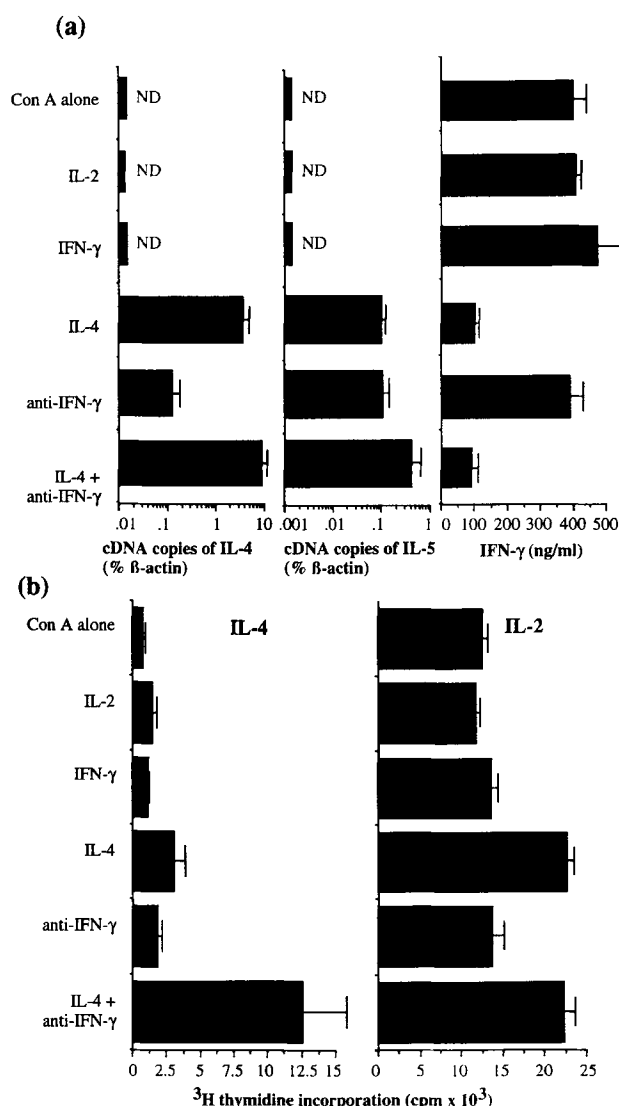


Fig. 3. The effect of different cytokines and cytokine blocking antibodies on (a) the differentiation of CD4⁺ T cells and (b) the proliferative response of CD4⁺ T cells to IL-2 and IL-4.

effects of IL-4 and IFN- γ on Th2-like cell development (1, 19, 28).

IL-4 suppressed IFN- γ secretion by restimulated lymphocytes (Fig. 3a), confirming earlier reports (28). However, addition of IFN- γ , or blocking of endogenous IFN- γ activity with anti-IFN- γ antibody, had no effect on the ability of the cells to make IFN- γ . Furthermore, while normal rat splenocytes are unable to proliferate in response to IL-4 alone, CD4⁺ T cells cultures in the presence of both IL-4 and anti-IFN- γ showed substantial levels of proliferation when cultured with recombinant IL-4 (Fig. 3b). This shows that Th2-like effector cells were generated in these cultures, since Th2 clones are highly responsive to IL-4. Cells cultured in IL-4 also had slightly increased responsiveness to IL-2.

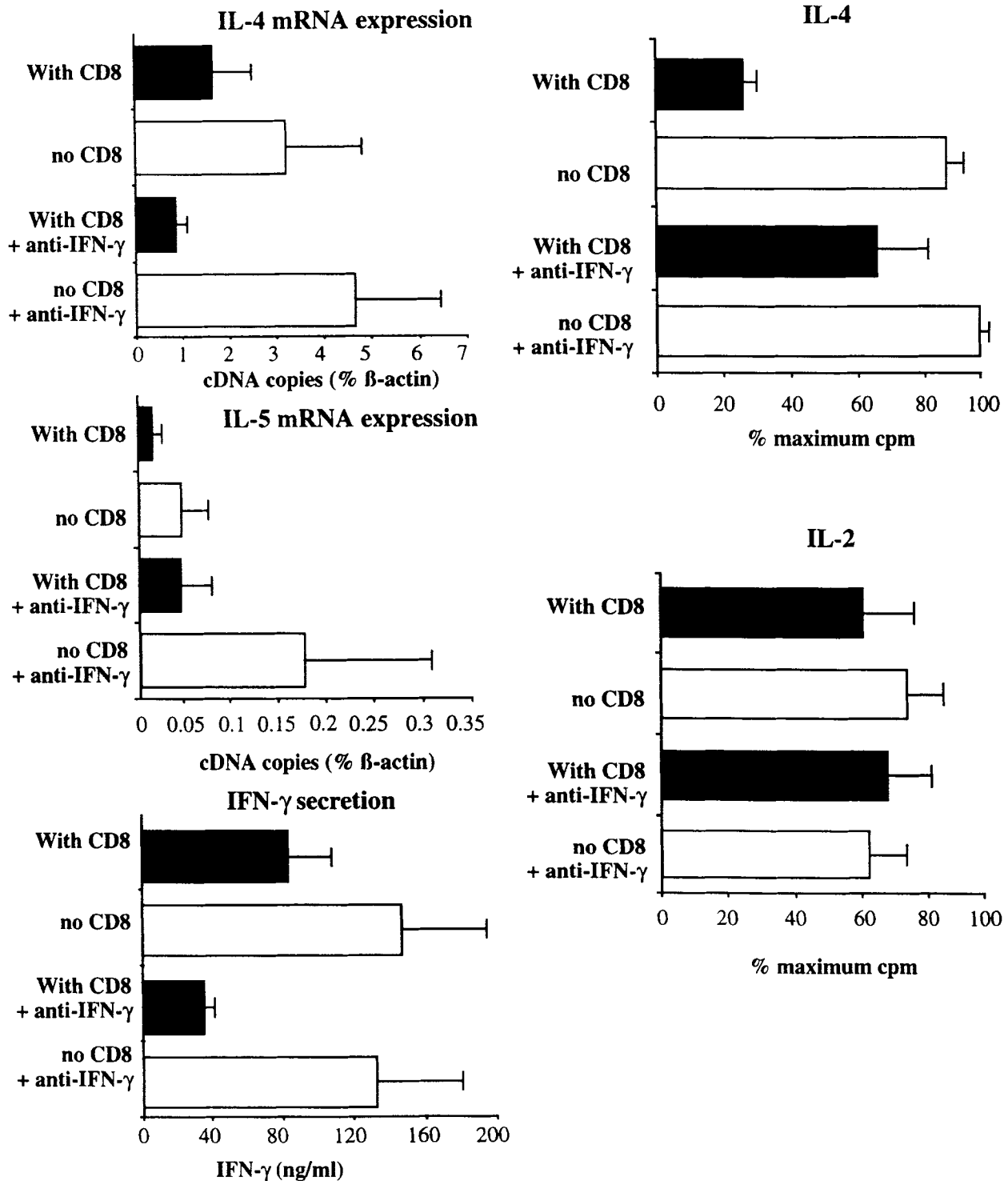


Fig. 4. The effect of CD8⁺ T cells on (A) CD4⁺ T cell differentiation and (B) Th2-like CD4⁺ T cell proliferation.

The effect of CD8⁺ T cells on the growth and differentiation of Th1 and Th2 CD4⁺ T cells

Evidence that CD8⁺ T cells might affect CD4⁺ T cell function was suggested by the observation that the amount of IFN- γ that could be produced by

CD4⁺, as well as CD8⁺ T cells, following stimulation with phytohemagglutinin (PHA) or PMA and ionomycin, following *in vivo* depletion of early-activated rat CD8⁺ T cells with ricin, was reduced by 5–10 fold (4), while their capacity to produce IL-4, IL-5 and IL-10 was increased over 10-fold (21). Furthermore, adoptive transfer of early-

activated CD8⁺ T cells to syngeneic animals enhanced PHA-stimulated IFN- γ production by over 1000-fold.

To determine whether CD8⁺ T cells influence cytokine production by cultured CD4⁺ T cells, experiments were performed in which CD8⁺ T cells were depleted from rat spleens by negative selection with anti-CD8 magnetic particles prior to culture. Mixed splenocytes from which CD8⁺ T cells had been removed were cultured with Con A, IL-4 + / - anti-IFN- γ . After 4 days, CD4⁺ T cells were isolated by positive selection with anti-CD4 magnetic agarose beads. The bound CD4⁺ T cells were >98% pure as judged by fluorescence-activated cell sorting (FACS) and were then stimulated with PMA and ionomycin and RNA extracted. The results (Fig. 4a) show that removal of CD8⁺ T cells increases IFN- γ as well as IL-4 and IL-5 production, indicating that they exert effects on both Th1 and Th2 cell development. CD8⁺ T-cell inhibition of Th1 and Th2 cytokine production was not affected by anti-IFN- γ blocking antibody, suggesting that these effects were not mediated by CD8⁺ T-cell derived IFN- γ . However, the ability of CD4⁺ T cells to proliferate in response to recombinant rat IL-4 was inhibited by CD8⁺ T cells, and here the effect of CD8⁺ T cells could be reversed with anti-IFN- γ (Fig. 4b). Thus CD8⁺ T cells can inhibit Th1 as well as Th2 CD4⁺ T cell-development. However, their inhibition of IL-4-driven Th2 T-cell growth may be the mechanism for CD8⁺ T cell inhibition of the IgE response. Alternatively, there may be more than one type of immunoregulatory CD8⁺ T cell, and this has been investigated below.

CD8⁺ T cell differentiation

The paradoxical effects of pre- and post-immunization CD8 depletion on the IgE response and the capacity of CD8⁺ T cells to block both Th1 and Th2 CD4⁺ T cell development suggested that there may be different populations of immunoregulatory CD8⁺ T cells. We therefore set out to study CD8⁺ T-cell differentiation. CD8⁺ T cells were isolated to >95% purity by positive selection with anti-CD8 antibody-coated magnetic particles and cultured with PMA, ionomycin and IL-2 in the presence or absence of either IL-4 or anti-IFN- γ . The cultures were subsequently supplemented with IL-2 and after 6 days the CD8⁺ T cells were washed and re-stimulated with PMA and ionomycin. As with CD4⁺ T cells, CD8⁺ T-cell expression of cytokine genes was affected by cytokines present in their microenvironment. However, the effects of IL-4 and IFN- γ on CD8⁺ T-cell differentiation differ from those of their CD4⁺ counterparts in a number of ways. As with CD4⁺ Th2 T-cell development, IL-4 appears to

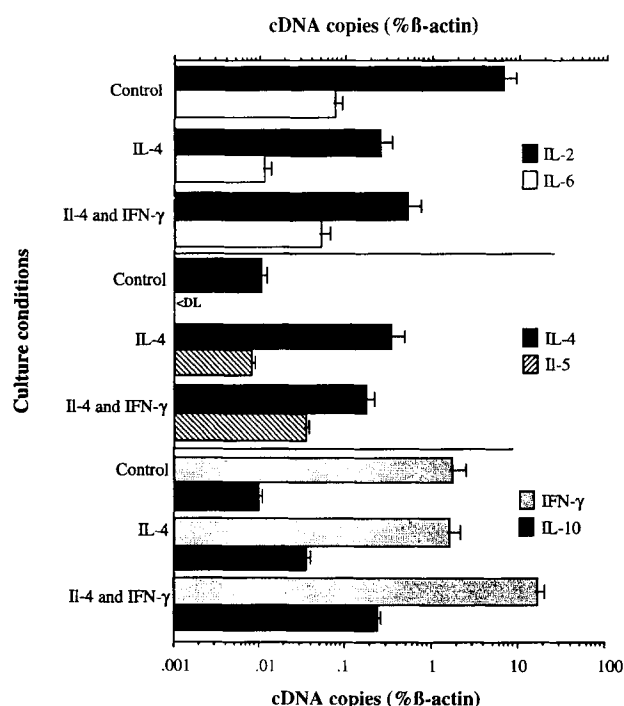


Fig. 5. The effects of IL-4 and IFN- γ on the cytokine profiles of cultured and re-stimulated CD8⁺ T cells.

be essential for CD8⁺ T-cell production of IL-4 and IL-5 (Fig. 5), but unlike CD4⁺ T cells, IL-4 inhibited IL-2 and IL-6 production. Again, unlike CD4⁺ T cells, expression of the genes for IL-10 and IFN- γ was increased when CD8⁺ T cells were cultured with IL-4 and anti-IFN- γ . Clearly CD8⁺ T cell differentiation does not completely parallel that of CD4⁺ T cells and the cytokine profiles of these cells presumably reflect their distinct functions in the immune system. How the functions of CD8⁺ T cells generated under these conditions differ and which of these regulate IgE must await further study and is the current focus of our research.

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