CD8 T Cells Inhibit IgE Via Dendritic Cell IL-12 Induction That Promotes Th1 T Cell Counter-Regulation

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CD8 T Cells Inhibit IgE Via Dendritic Cell IL-12 Induction That Promotes Th1 T Cell Counter-Regulation

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Th1 and Th2 cells are counterinhibitory; their balance determines allergic sensitization. We show here that CD8 T cell subsets break these rules as both T cytotoxic (Tc1) and Tc2 cells promote Th1 over Th2 immunity. Using IL-12−/−, IFN-γ−/−, and OVA257–264-specific Vα2Vβ5 TCR-transgenic mice, we have identified the key steps involved. OVA-specific IFN-γ−/− CD8 T cells inhibited IgE responses equivalent to wild-type CD8 T cells (up to 98% suppression), indicating that CD8 T cell-derived IFN-γ was not required. However, OVA-specific CD8 T cells could not inhibit IgE in IFN-γ−/− recipients unless reconstituted with naive, wild-type CD4 T cells, suggesting that CD4 T cell-derived IFN-γ did play a role. Transfer of either Tc1 or Tc2 Vα2Vβ5 TCR-transgenic CD8 T cells inhibited IgE and OVA-specific Th2 cells while promoting OVA-specific Th1 cell responses, suggesting a potential role for a type 1 inducing cytokine such as IL-12. CD8 T cells were shown to induce IL-12 in OVA257–264-pulsed dendritic cells (DC) in vitro. Furthermore, CD8 T cells were unable to inhibit IgE responses in IL-12−/− recipients without the addition of naive, wild-type DC, thus demonstrating a pivotal role for IL-12 in this mechanism. These data reveal a mechanism of IgE regulation in which CD8 T cells induce DC IL-12 by an IFN-γ-independent process that subsequently induces Th1 and inhibits Th2 cells. Th1 cell IFN-γ is the final step that inhibits B cell IgE class switching. This demonstrates a novel regulatory network through which CD8 T cells inhibit allergic sensitization. The Journal of Immunology, 2002, 168: 216–223.

In recent years, a number of different immunoregulatory processes have been defined. Th1 and Th2 responses are mutually inhibitory and can moderate potentially pathological allergic tissue reactions such as asthma. Inhibitory cytokines such as TGF-β (1) and IL-10 (2) produced by specific subsets of regulatory T cells also inhibit inflammatory immune responses (3). The mechanism for immune regulation of IgE Ab responses has yet to be fully defined. Following parenteral immunization with soluble Ags, it has been demonstrated that IgE responses require T cell help (4). CD4 Th2 T cells provide this help in the form of CD40 ligand, which activates B cells, and IL-4, which induces Ig class switching to IgE (5, 6).

CD8 T cells also can inhibit IgE responses (7, 8). CD8 T cells that inhibit IgE are sensitive to the toxic lectin ricin (9). Depletion of these cells in vivo using ricin or anti-CD8 mAb increases the capacity of CD4 T cells to produce IL-4 and decreases IFN-γ production (10, 11). Recently, these IgE inhibitory CD8 T cells have been cloned and found to express the αβ TCR and to be MHC class I restricted (12). Their capacity to inhibit IgE is unrelated to their ability to produce IFN-γ, but paradoxically their effect on IgE can be blocked by anti-IFN-γ mAb (12). CD8 T cells also regulate airway hyperresponsiveness in rats (13, 14). Presentation of soluble Ag (OVA) via MHC class I runs counter to the predominant APC pathway for soluble exogenous proteins in which Ag peptides are presented to CD4 T cells complexed with surface MHC class II molecules. However, there now is clear evidence that small but important amounts of such exogenous Ag are directed intracellularly to be presented via MHC class I (15, 16), and derived peptide MHC class I complexes can activate CD8 T cells. We have previously demonstrated that OVA-specific CD8 T cells can suppress IgE responses when adoptively transferred into wild-type recipients responding to OVA-alum immunization. We also determined that activating the regulatory potential of CD8 T cells was Ag specific, whereas their regulatory effects could influence IgE responses to commumized irrelevant Ag (17). This suggests a suppressive mechanism that, once activated to a single allergen, could have an impact on the generation of IgE response to other harmful allergens.

In the current study, we have investigated the mechanisms by which OVA-specific CD8 T cells inhibit IgE responses. Using IFN-γ−/− mice, we have shown that CD8 T cells do not need to produce IFN-γ to inhibit IgE, but that IFN-γ is required and is supplied by participating OVA-specific CD4 Th1 T cells. The immunoregulatory potential of CD8 T cells to inhibit IgE is also dependent on their ability to stimulate IL-12 production by APC that, in turn, activates Th1 cells. Thus, CD8 T cells are unable to inhibit IgE in IL-12−/− mice but can do so if IL-12−/− mice are reconstituted with wild-type (IL-12 competent) dendritic cells (DC).3

Materials and Methods
Mice and materials

Wild-type C57BL/6 mice (6–8 wk) were obtained from Harlan Olac (Bicester, U.K.). Several breeding pairs of OVA peptide-specific, class I-restricted Vα2Vβ5 TCR-transgenic mice (OT-I) were a kind gift from Dr. M. 3 Abbreviations used in this paper: DC, dendritic cell; Tc, T cytotoxic; PCA, passive cutaneous anaphylaxis; LN, lymph node.
CD11c (PE) to assess the number of DC present. Cells were also stained for

Generation of OVA-specific Tc1 (IFN-\(\gamma\)) and Tc2 (IL-4\(^+\)) ToxOVA-specific CD8 T cell lines

Because IgE responses are inhibited by IFN-\(\gamma\) and because CD8 T cells are strongly biased to produce IFN-\(\gamma\), we investigated the contribution of IFN-\(\gamma\) to CD8 T cell inhibition of IgE Ab responses by adoptive transfer of polarized Tc1 and Tc2 OVA-specific cell lines. Tc1 and Tc2 lines were generated in vitro with CD8 T cells from C57BL/6 (H2K\(^b\)) mice that have a transgenic V\(\alpha\)2V\(\beta\)5 TCR that specifically binds the OVA peptide 257–264 when complexed with anti-CD3, PMA (10 ng/ml), anti-CD28 (1 \(\mu\)g/ml), IL-4 (10 U/ml), and blocking anti-IL-12 (10 \(\mu\)g/ml) were added. Phenotypic assessment was by stimulation with OVA in PBS for 5 h followed by intracellular cytokine staining for IFN-\(\gamma\) and IL-4 and ELISA analysis of 24-h culture supernatant for secreted IFN-\(\gamma\), IL-4, and IL-10.

Intracellular cytokine staining

CD8 T cell cultures were washed thoroughly with PBS/1% FCS then resuspended in complete medium containing 5 \(\mu\)g/ml OVA peptide and 3 \(\mu\)M monensin at 10\(^5\) cells/ml for 5 h at 37°C. Cells were then washed and stained for FACS at 10\(^5\)/tube. Anti-CD4 or CD8 CyChrome-labeled surface marker Abs were then added and incubated for 15 min. Cells then were washed, incubated for 15 min with 250 \(\mu\)l of Perm/Fix solution (BD Pharmingen, San Diego, CA), then washed twice with 2–4 \(\mu\)l of Perm/Wash buffer (PBS/0.5% BSA/0.1% saponin; BD Pharmingen). Anti-IFN-\(\gamma\) FITC and anti-IL-4 PE were added at 1 \(\mu\)l/tube, mixed, and then incubated for 30 min at 18°C. Cells were then washed with Perm/Wash buffer as before and resuspended in 500 \(\mu\)l of 1% PFA. Cells were then analyzed using a BD Biosciences FACSCalibur flow cytometer.

Cytokine ELISAs

CD8 T cell cultures were washed thoroughly with PBS then resuspended in complete medium containing 5 \(\mu\)g/ml OVA at 10\(^5\) cells/ml for 24 h at 37°C. Supernatant from each well was stored at −30°C for ELISA analysis. Throughout, 50-\(\mu\)l volumes were used, and the assay was performed at 25°C. IFN-\(\gamma\), IL-4, IL-10, and IL-12 (p40) were measured using capture and detector Ab systems. Microtiter plates (Maxisorb; Nunc) were coated with detector Ab at 1 \(\mu\)g/ml in carbonate/bicarbonate (pH 9.6, 0.1 M) coating buffer overnight at 4°C and washed three times with PBS/0.05% Tween 20. Duplicate supernatant samples diluted 1/50 or greater in assay buffer (PBS/1% rat serum/0.5% Tween 20) were added. After 2 h, the plates were washed and biotinylated detector Ab at 1 \(\mu\)g/ml was added. After 2 h, the plates were washed and streptavidin-conjugated alkaline phosphatase was added at 1 \(\mu\)g/ml. After 2 h, the plates were washed, and \(p\)-nitrophenyl phosphate substrate diluted to 1 mg/ml in diethanolamine buffer (0.1 M) was added. After 1 h, absorbance was read at 405 nm in a plate reader (Molecular Devices, Crawley, U.K.), and the results were expressed as nanograms per milliliter by reference to a standard curve constructed using dilutions of recombinant cytokine.

Measurement of OVA-specific IgE by PCA

Passive cutaneous anaphylaxis (PCA) was used to measure IgE Abs, as this is the most reliable and reproducible method. It has been demonstrated that the only reaginic Ab active at the site of injection 48 h after skin transfer is IgE (18). Furthermore, the method correlates well with in vitro sensitization using rat basophil leukemia cells and monoclonal IgE. In addition, it provides evidence that the Abs measured are functional. PCA is not affected by high IgG Ab titers or changing Ab affinity as can be ELISA. Mouse serum OVA-specific IgE titers were measured by PCA in Wistar rats (Harlan Olac). Serial 4-fold dilutions of serum from 1/8 to 1/2048 in assay diluent (PBS/1% rat serum/0.5% Tween 20) were added. After 30 min, we investigated the contribution of IFN-\(\gamma\) to CD8 T cell inhibition of IgE Ab responses by adoptive transfer of polarized Tc1 and Tc2 OVA-specific cell lines. Tc1 and Tc2 lines were generated in vitro with CD8 T cells from C57BL/6 (H2K\(^b\)) mice that have a transgenic V\(\alpha\)2V\(\beta\)5 TCR that specifically binds the OVA peptide 257–264 when complexed
to MHC class I H2Kb (19). Tc1 lines were polarized by culture of Vα2Vβ5 TCR CD8 T cells for 3 days with plate-bound anti-CD3 and IL-12. Tc2 lines with plate-bound anti-CD3, anti-CD28, PMA, IL-4, and anti-IL-12. When analyzed for intracellular cytokine production following stimulation with OVA257–264 for 5 h, 56% of Tc1 cells were found to produce IFN-γ alone, 0.2% produced both IFN-γ and low levels of IL-4, and no Tc1 cells were detected that produced IL-4 alone. Conversely, 36% of Tc2 cells produced IL-4 alone, 0.3% produced both IL-4 and low levels of IFN-γ, and 0.1% produced IFN-γ alone (data not shown). The cytokine profile of both subsets was confirmed by stimulation of the polarized Tc1 and Tc2 cells with OVA257–264 for 24 h and analysis of secreted cytokines in supernatants by ELISA. Tc1 cells produced 276 ng/ml IFN-γ with no detectable IL-4 or IL-10, whereas Tc2 cells produced 5.5 ng/ml IL-4, 14 ng/ml IL-10, and very low levels of IFN-γ at 1.4 ng/ml.

**Tc1 cells are more effective inhibitors of IgE responses than Tc2 cells**

To determine the relative IgE inhibitory potential of OVA-specific Tc1 vs Tc2 cells, numbers varying from 10^5 to 10^6 were adoptively transferred into wild-type recipients immunized simultaneously with OVA in alum (Fig. 1). Control groups received naive, unpolarized Vα2Vβ5 CD8 T cells or OVA-alum immunization alone. Both control groups made an OVA-specific IgE response that peaked at day 7 with a titer of over 1/512, declining to basal levels (1/8) by day 21. However, transfer of in vitro polarized 10^6 OVA257–264-specific Tc1 or Tc2 cells inhibited the IgE response 64- and 32-fold at day 7 (Fig. 1a) (p < 0.01). Dose response showed that transfer of 10-fold less (10^5) Tc1 or Tc2 cells also inhibited IgE by 12- (p < 0.01) and 8-fold (p < 0.05) at day 7. A difference in the inhibitory abilities of Tc1 and Tc2 cells was observed when 10^4 cells were given (Fig. 1b). At this cell number, Tc1 cells still inhibited IgE responses 16-fold (p < 0.01), whereas Tc2 cells could only exert an ~2-fold reduction in IgE (p > 0.05).

Adoptive transfer of 10^3 and 10^4 anti-OVA Tc1 or Tc2 cells did not result in significant inhibition (p > 0.05). Importantly, significant inhibition was still observed from 10^3 Tc2 cells that barely produced detectable levels of IFN-γ. Further, levels of OVA-specific IgG1 were unaffected by adoptive transfer of all doses of Tc1 or Tc2 CD8 T cells, showing that CD8 cell inhibition was isotopy specific for these two Th2-dependent Ab classes (data not shown).

**OVA-specific CD8 T cells promote Th1 and inhibit Th2 OVA-specific CD4 T cell responses**

Because CD8 T cells inhibited IgE, we also determined their effect on the concomitant CD4 T cell response using a similar adoptive transfer protocol using 10^6 Tc1 or Tc2 cells. CD4 T cells and APC were purified from day 7 mice immunized with OVA-alum to induce IgE that had received Vα2Vβ5 Tc1 (Fig. 2a) or Vα2Vβ5 Tc2 CD8 T cells (Fig. 2b), or no CD8 T cells (Fig. 2c), and were cultured with 100 μg/ml OVA. The generation of intracytoplasmic cytokines (IL-4 and IFN-γ) in the CD4 T cells was determined 6 days later following restimulation with PMA and ionomycin. In the positive control animals that received OVA-alum without CD8 T cells, the percentage of OVA-specific LN CD4 Th2 cells (IFN-γ–IL-4+) and Th1 cells (IFN-γ–IL-4–) following culture with OVA were 23 and 0.4% respectively (Fig. 2c). In recipients of Tc1 cells, the percentage of Th2 cells was reduced to 1.1%, while Th1 cells were increased to 35% (Fig. 2a). Similarly, although not as dramatically, the percentage of Th2 cells was reduced to 3.7% in recipients of Tc2 cells, while the percentage of Th1 cells was increased to 11% (Fig. 2b). Thus, both Tc1 and Tc2 OVA-specific CD8 regulatory T cells, when given at the induction of the IgE response with OVA-alum, promoted development of specific Th1 cells in recipients that may have been involved in the inhibition of the CD4 Th2 (IL-4-dependent) responses, perhaps by producing IFN-γ, that lead to a decrease in IgE production by B cells.

**FIGURE 1.** Inhibition of IgE by Tc1 and Tc2 CD8 T cells. a, In vitro primed Vα2Vβ5 TCR Tc1 (△) or Tc2 (▽) cells were transferred to groups of five wild-type (C57BL/6) mice (10^6 per recipient) immunized i.p. concurrently with 100 μg of OVA-alum. Control mice (n = 5) were given 10^6 unstimulated OVA257–264-specific Vα2Vβ5 TCR CD8 T cells and were immunized with 100 μg of OVA-alum i.p. (○) or were immunized with OVA-alum alone (●). The results are representative of three independent experiments. b, Different numbers (10^7–10^8) of in vitro primed Vα2Vβ5 TCR Tc1 (△) and Tc2 (▽) cells were adoptively transferred i.p. to groups of five wild-type (C57BL/6) mice immunized concurrently with 100 μg of OVA-alum i.p. Control mice were given 10^6 CD8 T cells from unstimulated OVA257–264-specific Vα2Vβ5 TCR-transgenic mice and were immunized with 100 μg of OVA-alum i.p. (filled bar), or were immunized with OVA-alum alone (open bar). The time of immunization and cell adoptive transfer are indicated by the arrow. IgE Ab levels compared with controls are represented: *, p < 0.05; **, p < 0.005.
CD8 T cells inhibit OVA-specific serum IgE responses in wild-type but not IFN-γ−/− mice

Although IFN-γ is noted for its ability to inhibit IgE responses, the capacity of Tc2 CD8 T cells to suppress IgE suggests that IFN-γ may not be required for inhibition by CD8 T cells because Tc2 cells made practically no IFN-γ. However, this small amount of IFN-γ could have inhibited IgE, or IL-12 that was produced by APC could have induced IFN-γ in the Tc2 CD8 T cells after transfer. Therefore, we determined whether CD8 T cells from IFN-γ−/− mice could inhibit the OVA-specific IgE response. We previously showed that CD8 T cells collected at day 21 from mice immunized with 100 μg of OVA-alum inhibited IgE responses when adoptively transferred to naive mice that were then similarly immunized (17). These will henceforth be called “OVA-primed day 21 CD8 T cells.” OVA-primed day 21 CD8 T cells from IFN-γ−/− mice inhibited the OVA-specific IgE response as effectively as CD8 cells from wild-type mice (Fig. 3a). However, promotion of OVA-specific Th1 and inhibition of OVA-specific Th2 cells suggested that there might be a role for IFN-γ in the regulatory process. Indeed, a requirement for IFN-γ was demonstrated when day 21 OVA-primed CD8 T cells were unable to inhibit IgE in IFN-γ−/− mice (Fig. 3b), but could do so in IFN-γ−/− mice that were constituted with CD4 T cells isolated from naive wild-type mice that may have produced essential IFN-γ (Fig. 3c). Thus, CD8 T cell inhibition of IgE is independent of CD8 T cell-derived IFN-γ but does require IFN-γ production by CD4 T cells.
in supernatants from unpulsed DC, and <2 ng/ml was detected at either time point in supernatants from Va2Vβ5 T cells cultured with irrelevant peptide-pulsed DC. Thus, inhibitory Ag-specific CD8 T cells can induce IL-12 production in DC following stimulation with specific Ag.

**CD8 T cell-mediated IgE inhibition depends on DC production of IL-12**

IL-12-/- mice were used (20) to determine the possible contribution of IL-12 in the inhibition of IgE responses by DC and CD8 T cells in vivo. Adoptive transfer of 10⁵ Tc1 and Tc2 Va2Vβ5 TCR CD8 T cells failed to inhibit IgE responses in IL-12-/- compared with wild-type mice (Fig. 5, group B). The importance of DC-derived IL-12 was indicated further by reconstitution of IL-12-/- mice in a dose-dependent fashion with DC (10⁷–10⁵ DC per mouse) from naive (IL-12-competent) mice (Fig. 5b, groups C–F). An important control was adoptive transfer of 10⁵ DC from naive (IL-12-competent) mice with 10⁶ naive DC8 T cells (Fig. 5b, group A) to IL-12-/- mice. No inhibition was observed in this group, demonstrating that DC alone could not inhibit the IgE response. These data show that IL-12 is an essential element of the regulatory CD8 T cell pathway.

**The role of CD4 T cells in CD8 T cell-mediated IgE regulation**

Although we had shown that OVA-specific CD8 T cells induced IL-12 and that a shift from a Th2 to a Th1 dominant response was associated with inhibition of IgE, the relationship among DC, CD4 T cells, and the cytokines they produce in CD8 T cell suppression of IgE was unclear. For this purpose, IFN-γ-/- and wild-type mice were used to generate Th1-like cells. Intracellular cytokine analysis of Th1 cells from wild-type mice revealed that 50% stained positive for IFN-γ, 1.2% stained positive for IL-4, and 19% stained positive for IL-2 (Fig. 6a). In contrast, of Th1-like cells from IFN-γ-/- mice, only 0.4% stained positive for IFN-γ and 1.7% stained positive for IL-4, but more (32%) were positive for IL-2 (Fig. 6a). We then adoptively transferred IFN-γ-/- and IL-12-/- recipient mice with either naive CD4 T cells (Fig. 6b, groups C and D), OVA-primed day 21 wild-type (Fig. 6b, groups E and F), or IFN-γ-/- (Fig. 6b, groups G and H) Th1 cells. As before (Fig. 3c), coadoptive transfer of naive wild-type CD4 T cells, with day 21 OVA-primed CD8 T cells, into IFN-γ-/- recipient mice reconstituted inhibition of the IgE response by up to 16-fold (Fig. 6b, groups C and D). However, inhibition of IgE did not occur in IL-12-/- recipients. This finding demonstrated that the mechanism by which CD8 T cells inhibit the CD4 Th2 response is dependent on IL-12. Coadoptive transfer of OVA-specific Th1 cells with day 21 OVA-primed CD8 T cells resulted in suppression in both IFN-γ-/- and IL-12-/- recipient mice by between 8- and 16-fold (Fig. 6b, groups E and F), demonstrating that, if generated before transfer, Th1 cell inhibition of IgE was independent of IL-12. In other words, IL-12 was only required to generate Th1 cells. However, suppression was not observed in either IFN-γ-/- or IL-12-/- recipient mice adoptively transferred with day 21 OVA-primed CD8 T cells together with IFN-γ-/- Th1 cells (Fig. 6b, groups G and H), suggesting that DC-derived IL-12 was the induced factor that was vital for IgE suppression.

The ability of CD8 T cells to inhibit IgE was not shared by CD4 T cells, as day 21 OVA-primed CD4 T cells from wild-type or IFN-γ-/- mice (Fig. 6c, groups C and D) transferred to wild-type recipients were unable to suppress the IgE response compared with control animals (Fig. 6c, group A) or mice that received day 21 OVA-primed CD8 T cells (Fig. 6c, group B), thereby confirming that the IgE regulatory pathway described here is a unique property of the CD8 T cell.

**Discussion**

We investigated the mechanism of CD8 T cell regulation of IgE using OVA-specific Va2Vβ5 TCR transgenic T cells and IFN-γ-/- and IL-12-/- mice. At the outset, CD8 T cell IFN-γ was considered the most likely candidate for CD8 T cell inhibition of IgE, because IFN-γ inhibits Th2 T cell growth (21) and B cell IgE class switching (6). However, the experiments described in this work suggest that this is not the case. Our results show that OVA-specific CD8 inhibitory T cells interact with DC independently of IFN-γ to induce IL-12 that, in turn, promotes the generation of OVA-specific Th1 cells that inhibit Th2-dependent IgE class switching via production of IFN-γ. The ability of CD8 T cells to activate this pathway is unique and is not shared by CD4 T cells.

![Figure 5](http://www.jimmunol.org/) Inhibition of IgE in IL-12-/- mice. In vitro primed CD8 Tc1 (10⁵) and Tc2 (10⁵) cells from Va2Vβ5 TCR-transgenic mice were adoptively transferred to groups (n = 5) of IL-12-/- mice (● and ○, respectively) or wild-type mice (▲ and □, respectively) immunized concurrently with 100 μg of OVA-alum i.p. Control IL-12 knockout mice were similarly immunized and given 10⁶ CD8 T cells from naive donors (▲). The time of immunization and cell adoptive transfer are indicated by the arrow. The ability of CD8 T cells to inhibit IgE was not shared by CD4 T cells, as day 21 OVA-primed CD4 T cells from wild-type or IFN-γ-/- mice (Fig. 6c, groups C and D) transferred to wild-type recipients were unable to suppress the IgE response compared with control animals (Fig. 6c, group A) or mice that received day 21 OVA-primed CD8 T cells (Fig. 6c, group B), thereby confirming that the IgE regulatory pathway described here is a unique property of the CD8 T cell.
were cultured for 3 days in Th1 polarizing conditions. Intracellular cytokine production was isolated from OVA-primed day 21 wild-type or IFN-γ/H9253 mice. Thus, this is the first time that a type-2 T cell has been shown to promote a Th1-type response. The significance of these observations is that activation, possibly of any CD8 T cell possessing the appropriate TCR, has the potential to influence the differentiation of co-responding CD4 T cells, and therefore the development of the ensuing immune response. This finding may have potential application to targeted vaccination to prevent allergies.

**FIGURE 6.** The role of CD4 T cells in CD8 IgE regulatory pathway. a. Generation of OVA-specific IFN-γ+/−Th1-like CD4 T cells. CD4 T cells isolated from OVA-primed day 21 wild-type or IFN-γ−/− mice were cultured for 3 days in Th1 polarizing conditions. Intracellular cytokine production was measured for IFN-γ and IL-4 and IL-2 and IL-4. b. The ability of CD4 T cells to regulate IgE was dependent on their ability to make IFN-γ. Mice received 10⁵ CD8 T cells from OVA-primed day 21 wild-type mice, together with either 2 × 10⁵ naive CD4 T cells from wild-type mice (groups C and D), 2 × 10⁵ OVA-specific Th1 cells from wild-type mice (groups E and F), or 2 × 10⁵ OVA-specific Th1 cells from IFN-γ−/− mice (groups G and H). OVA-specific IgE were measured levels 7 days following immunization and adoptive transfer into either IFN-γ−/− (hatched bars) or IL-12−/− (filled bars) recipient mice. Control groups received OVA-alum immunization only (groups A and B). c. CD4 T cells are unable to activate the same pathway as CD8 T cells. Mice received 10⁵ OVA-specific Th1 cells from wild-type (group C) or IFN-γ−/− mice (group D). Control groups received OVA-alum immunization only (group A) or 10⁵ CD8 T cells from OVA-primed day 21 wild-type mice (group B). IgE Ab levels compared with controls are represented: *, p < 0.05; **, p < 0.005. This experiment is representative of two independent experiments.

Surprisingly, Tc2 Vα2Vβ5 TCR CD8 T cells also inhibited IgE. That a type 2 cell could induce a type 1 response runs counter to our view of immune regulation. Tc1 and Tc2 cells both promoted Th1 cell development and inhibited generation of Th2 CD4 T cells. Thus, this is the first time that a type-2 T cell has been shown to
MECHANISM OF CD8 IgE REGULATION

Both IL-18-dependent (LPS-induced shock) and -independent (in vivo Staphylococcus aureus, enterotoxin-B) induction of IFN-γ have been described (35).

Our results demonstrate that Ag-specific, cognate communication between the CD8 T cell and DC is essential (17) for inhibition of IgE. The molecules induced by this cognate interaction that actually inhibit the IgE B cell response (IL-12 and IFN-γ) have yet to be defined but are not Ag specific. This contrasts with earlier descriptions of IgE regulatory factors before the αβ TCR was discovered, which appeared to be Ag specific (36, 37). CD8 T cells require OVA peptide-MHC activation on DC to stimulate IL-12, but because IL-12 acts nonspecifically, if other Ag also are present and are stimulating a primary IgE response, they too will be inhibited (17). This has important consequences for therapy of allergic disease, because it should only be necessary to stimulate CD8 T cells that recognize a single peptide to inhibit the IgE response not only to this peptide but also to other peptides generated from that Ag, and even to other Ag that are also present.

In contrast to CD4 T cells, CD8 T cells can be activated by TCR ligation alone (38, 39) and do not require costimulation for priming (40, 41). Indeed, CD8 T cells primed in vivo with low affinity peptides could kill efficiently in vitro (42). Strategies for facilitating such presentation of Ag to TCR, for example as an approach to treating allergies, could include DNA immunization (43, 44) and cationic lipid encapsulation of antigenic peptide that directly fuses with the APC cell membrane, thus introducing Ag into the cytosol and therefore the MHC class I processing pathway. Cross-priming in which soluble Ag enters the MHC class I pathway has been well established by a number of investigators (15, 45–47). Indeed, as whole OVA was able to induce IgE inhibitory CD8 T cells in both rats (12) and in mice as shown in this study, it is possible that one of the contributing factors to the genetic predisposition of an individual to allergies (atopy) are defects in MHC class I cross-priming, leading to a reduced ability to activate IgE-inhibiting CD8 T cells. The pathway of IL-12 activation described may be crucial for determining the apparent constitutive set point of the immune response that ensures dominance of protective immunity to newly encountered Ag by promoting Th1 over Th2 responses. This would prevent allergic sensitization by inhibiting Th2 responses and thus consequent IgE production. This pathway may contribute to outgrowing allergies in childhood. We predict that this pathway may be involved in ongoing allergies. Thus, targeting this pathway might inhibit allergic sensitization in infants and could attenuate allergic immune responses in adults.

Our findings on the CD8 T cell-DC IL-12 pathway of inhibiting IgE responses bring molecular definition to the important processes that down-regulate IgE. Investigation of immune regulation has long focused on mechanisms of inducing IgE responses, such as Th2 cells, their cytokines IL-4 and IL-13, and cell surface co-stimulatory CD40/CD40-ligand-dependent signaling processes. By contrast, down-regulation of IgE, which has immense potential clinical application, has received less attention. Although it was known that CD8 T cells and IFN-γ were involved, the precise steps were unclear. This study details a novel mechanism of such immune down-regulation, explaining the role of the inhibitory CD8 T cell, which stimulates DC to produce IL-12 that activates CD4 Th1 cells to produce IFN-γ, which inhibits IgE-producing B cells.

Acknowledgments

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References


