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Generation of Rat MHC Class-I-Restricted Ovalbumin-Specific IgE Inhibitory CD8⁺ T Cell Clones

Key Words

CD8⁺ T cell
MHC class I
Clones
IgE
Immune regulation

Abstract

Allergic disease presents an increasing health problem in industrialised countries. Despite improved diagnosis and drug therapy, the number of fatalities from asthma and anaphylaxis continues to rise. A key element in the aetiology of the allergic response to inert protein antigens is an inherent imbalance in the atopic allergen specific CD4⁺ T cells, resulting in a dominance of the pathological, IL-4 producing, T helper 2 phenotype which favours B cell IgE production. Previous work from our laboratory, and elsewhere, has established that CD8⁺ T cells play an important role in regulating the magnitude and duration of IgE responses and may be vital for the restoration of IgE immunologic homeostasis in 'normal' non-atopic humans and low/medium responder rodents. We have found that CD8⁺ T cell depletion during a critical period after immunization produces a high and persistent IgE response in otherwise IgE hyporesponsive animals. We and others have demonstrated that small, but important, quantities of soluble antigen can enter the MHC class I pathway and, using ovalbumin (OVA)-transfected antigen-presenting cells, have succeeded in cloning OVA-specific, MHC class I-restricted rat α/β T cell receptor (TcR) positive CD8⁺ T cells. These cells universally secrete IFN- γ (5–69 ng/ml) and IL-2 (7.6–37.0 U/ml) and occasionally IL-4 (16.0–81.0 IU). When adoptively transferred, 1×10^6 of these cloned cells inhibit IgE production in vivo by as much as 50-fold and this effect can be titrated down to 1×10^3 cells. The IgE regulatory activity of the clones appears unrelated to their cytolytic potential or to their capacity to make IFN- γ .

Results and Discussion

We have previously reported that the intraperitoneal immunization of Lister Hooded rats with the non-replicating antigen ovalbumin (OVA) complexed to the IgE-specific adjuvant aluminium hydroxide results in the activation of a population of CD8⁺ T cells in the localised lymphoid tissue which suppress IgE when adoptively transferred to syngeneic animals [1]. Moreover, in vivo depletion of CD8⁺ T cells at specific time points results in a marked increase in

IgE (>46-fold). The present study sought to define the nature of IgE inhibitory CD8⁺ T cells by generating CD8⁺ T cell clones from the posterior mediastinal and parathymic lymph nodes of day 14 OVA-alum-immunized PVG RT7^b rats. At this time point, in vivo CD8 depletion is known to enhance the IgE response and an activated population of CD3⁺ T cells could be distinguished that were CD8^{high} and CD25^{high}. When purified, these cells exhibited a strong proliferative response to OVA-transfected APCs and the responding CD8⁺ T cells were MHC class I restricted – a

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1018–2438/97/1133–0279 \$12.00/0

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monoclonal antibody specific for a monomorphic determinant on MHC class I effected a significant decrease in the OVA-specific proliferative response of the purified CD8+ T cells. A monoclonal antibody specific for a monomorphic determinant on MHC class II had no effect, even at ten-fold higher concentration.

CD8+ T cells from the draining LN of day 14 OVA/alum-immunized rats were placed at limiting dilution in order to assess the precursor frequency of the responding cells. The frequency of OVA-specific CD8+ T cells was much lower than for the corresponding CD4+ T cells purified at the same time and cultured under comparable conditions (approximately 1:4298 CD8 and 1:722 CD4 responders). This suggests that most of the exogenous OVA introduced into the peritoneum is directed via the conventional MHC class II pathway, to OVA-specific CD4+ T cells. The OVA-specific CD8+ T cell clones generated could be separated into two groups based upon their cytokine secretion following antigen stimulation. All of the clones secreted IFN- γ , IL-2 and a small number also secreted IL-4. The existence of functionally distinct subsets within the CD8+ T cell pool is well recognised, with evidence from transgenic mouse and rat systems [2, 3]. It has been proposed from *Mycobacterium leprae* studies that non-cytotoxic human CD8+ T cell clones secrete IL-4 [4]. However, when the cytolytic potential of six of the OVA specific clones was tested with OVA-transfected splenocyte targets, there was no correlation between IL-4 secretion and a lack of cytotoxic activity, with five out of the six clones tested displaying efficient killing of targets.

The OVA specific CD8+ T cells clones generated following the peritoneal introduction of OVA-alum were $\alpha\beta$

TCR+, $\gamma\delta$ TCR-, CD8+, CD4-, CD3+. The IgE immunoregulatory potential of six of the clones was tested by adoptive transfer of 1×10^6 cells into CD8-depleted syngeneic recipients. All of the clones transferred effected a significant decrease in OVA-alum induced IgE in vivo without affecting the levels of OVA-specific IgG, and in the case of clone OACD8.45, this effect could be titrated down to as few as 1×10^3 cells. We looked for a correlation between the cytolytic activity of the clones and their immunoregulatory potential as several recent reports have intimated that cytotoxic lymphocytes elicited during an on going immune response against a soluble antigen may reduce the magnitude of the antigenic stimulus by killing APC targets [5]. However we could find no significant correlation between IgE levels and cytotoxicity. We also determined whether there were direct links between in vitro IFN- γ production and IgE immunoregulatory potential as there have been precedents for this thesis in recent reports detailing immune deviation of the atopic T cell response away from the pathogenic Th2 phenotype [6]. Again we found no significant correlation. Hence, it seems likely that the mechanisms underlying the IgE-specific immunoregulatory activity of CD8+ T cells are complex and multifaceted, where several factors including cytokine secretion, cytotoxicity, cell motility and survival could combine to mediate the in vivo effect. These mechanisms are currently under investigation in our laboratory.

Acknowledgment

This work is supported by a grant from the Wellcome Trust.

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