

REVIEW

The role of CD8⁺ T cells in the regulation of IgE

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Introduction

The ability to mount an IgE response to common environmental allergens (atopy) [1] is influenced by genetic factors. The progeny of atopic parents are more likely to produce IgE antibodies than their peers [2]. In the same way inbred strains of mouse [3] and rat [4] can be broadly divided into high and low IgE responders. The reason that non-atopic individuals and low IgE responder strains of rat and mouse do not make IgE antibodies to environmental antigens may be due to a number of factors: (i) the failure of the immune system to recognize small quantities of allergen [5]; (ii) the generation of IgE specific suppressor cells [6] or; (iii) to an inability to generate appropriate T-cell help [7]. Whilst it is possible that atopic individuals process antigen in a different way to their non-atopic counterparts it is unlikely that the first explanation is correct—nearly all humans make IgG antibodies against grass pollen and house dust mite [8], although these are of the IgG1 rather than the IgG4 subclass. Furthermore, T-cells from non-atopic individuals do proliferate in response to these allergens [9,10]. In experimental animals, immune responses to small quantities of antigen have been described in low IgE responder strains of rat which are reported to produce IgG antibodies and antigen-specific IgE-specific suppressor-T-cells [11]. Many infants are capable of mounting an IgE response when first exposed to an antigen [12] although such responses are usually short-lived [13] and the majority of adults, too, can make an IgE response if immunized in the presence of aluminium hydroxide [14] but again such responses are short lived.

T-cell control of IgE

Since the early 1970s it has been known that IgE responses require T cell help. Initial evidence was obtained by Tada *et al.* [15], and confirmed by others [7,16]. Tada showed that neonatally thymectomized rats were unable to mount a primary IgE antibody response

unless reconstituted with thymocytes from syngeneic animals. More recently Katona *et al.* have shown that anti-CD4 antibody suppresses the ongoing IgE response in parasitized mice [17]. As well as providing help for IgE B cells, T cells have also been shown to be capable of suppressing both primary and secondary IgE responses [18,19].

The role of cytokines in IgE regulation

Thus T-cells appear to fulfil two opposing functions in IgE regulation. Their regulatory effects are mediated, at least in part, by specific cytokines. High levels of interleukin 4 (IL-4) potentiate IgE synthesis *in vitro* [20,21] and interferon γ suppresses it (IFN γ) [22]. Both anti-IL-4 antibody [23,24] and IFN γ [25] suppress both ovalbumin and *Nippostrongylus braziliensis* induced IgE responses *in vivo*. In *N. braziliensis* infestation, IgE levels spontaneously decline following worm expulsion but similar effects were also seen with anti-IL-4 and IFN γ in *Heligmosomoides polygyrus* infested mice—where the worm is not expelled [26]. The secondary IgE response produced with antigen and Al(OH)₃ was largely, but not completely, inhibited with anti-IL-4 [27].

Differential cytokine production by T-cell subsets

IL-4 is produced by CD4⁺ T-cells and by Fc ϵ RI bearing non-T- non-B-cells which are believed to be mast cell progenitors [28]. IFN γ is produced by T-cells and macrophages. Analysis of murine T-cell clones [29] has shown that these cells can be sub-divided into those that make IFN γ and IL-2 (TH-1) and those that make IL-4 and IL-5 (TH-2) although it is now clear that there are a number of other subpopulations of CD4⁺ T-cells with different cytokine profiles [30]. Less is known about murine CD8⁺ T-cells in this regard and there are fewer CD8⁺ T-cell clones but those described so far exhibit a similar pattern of cytokine restriction to TH-1 CD4⁺ T-cells [31] although some human alloreactive CD8⁺ T-cell clones secrete IL-4 [32]. Antigen-specific T-cells that suppress IgE, generated in rats following inhalation of antigen, are of the CD8 phenotype [33].

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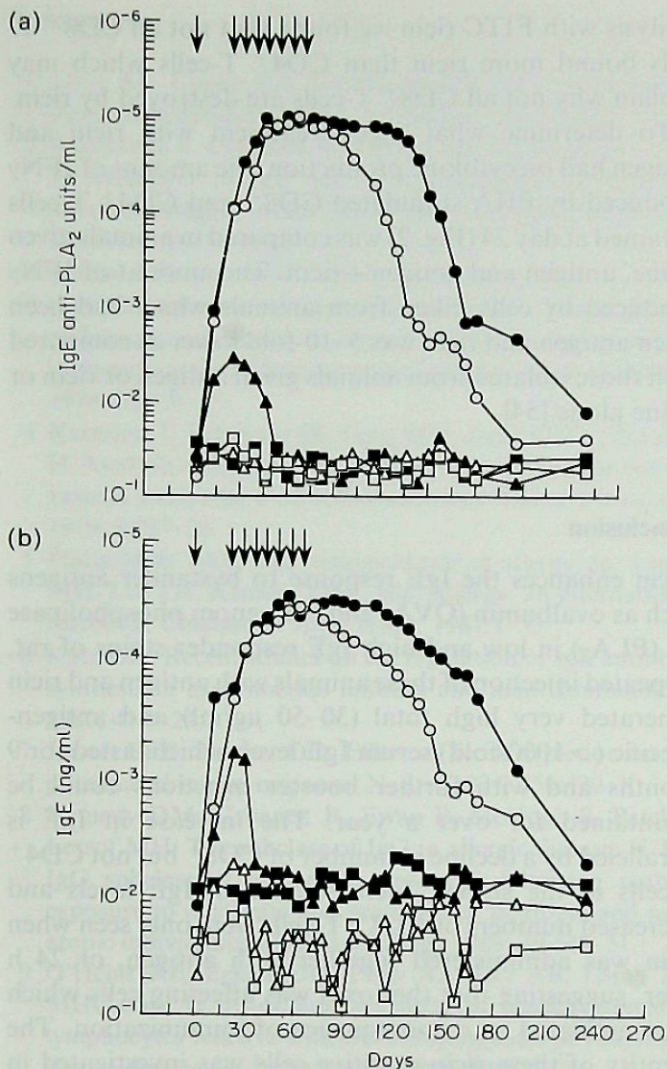


Fig. 1. Generation of a long lived IgE response by repeated injection of ricin + antigen. The levels of IgE anti-PLA₂ (a) and total serum IgE (b) in both Wistar (open symbols) and Brown Norway rats (closed symbols). The primary and booster injections of PLA₂ and ricin are shown by the arrows. The standard deviation has been omitted in the interests of clarity but did not exceed 18% of the mean for the total IgE or 17% or the mean for PLA₂-specific IgE. The results for animals given ricin alone were identical to those given saline and so are not shown. Reproduced from [47], with permission. ■, □, saline. ▲, △, PLA₂. ●, ○, + ricin.

Castor bean allergy

While genetic factors may render atopic humans and some strains of rat and mouse better able to produce IgE than others, a number of other factors may influence IgE responses. These include viral infection [34], parasitic infestation [35], cytotoxic drugs [36,37], sub-lethal X-ray irradiation [38] and plant lectins [39]. However, the most potent sensitizer so far described is the castor bean, *Ricinus communis*, which has caused outbreaks of asthma

in exposed populations throughout the world [40,41]. In both Port Sudan [42] and Marseille [43] we found that the frequency of atopy, as defined by a positive radioallergo-sorbent test (RAST) to one or more of the five most potent allergens in the region, was no different in those who were allergic to castor bean than in unexposed control subjects. The central question raised by these studies was why it was possible for castor bean to induce IgE responses in non-atopic individuals? Indeed it is believed that nearly all those who are exposed to castor bean dust become sensitized.

The effect of ricin on the IgE and IgG antibody responses

Preliminary experiments using a crude aqueous castor bean extract showed that IgE responses could be produced both to the major castor bean allergen, albumin [44], and to concomitantly administered ovalbumin in a moderate IgE producer strain, the Hooded Lister rat [45]. This effect was lost if the extract was heated. The most notable heat labile material in castor bean is the toxic lectin ricin and the IgE potentiating effect of castor bean could be reproduced when pure ricin was given together with OVA or PLA₂ [45,46]. Although IgG antibodies were produced in all animals, ricin neither enhanced nor suppressed the IgG or subclass of IgG antibody produced. Furthermore, it had no effect on total serum IgG levels. The effect of ricin on IgE response was compared in different strains of rat [47]. Ricin enhanced IgE production to the same levels in low (Wistar), moderate (Hooded Lister & PVG) and high (Brown Norway) IgE responder rat strains.

The IgE response could be prolonged for 9 months or more by repeated immunization with ricin and PLA₂ (Fig. 1) and the levels of IgE reached 30–50 µg/ml IgE [47]. The serum IgE levels found in these animals are comparable to those seen in high IgE responder rats infested with helminth parasites such as *N. braziliensis*. But even with parasitic infestation it is reported that there is considerable variation in IgE levels found in different rat strains. Brown Norway rats can have mean total serum IgE levels nearly 100 times higher than low responder strains of rat [48]. Induction of IgE tolerance by administration of soluble antigen requires doses 100–1000-fold lower in low IgE responders strains compared with high IgE responder strains [49]. Even with Al(OH)₃ picogram doses of antigen must be used in the low IgE responder Wistar rat to avoid activating the antigen-specific IgE suppressor mechanisms which normally operate [50]. With ricin, 1 µg or more of antigen may be used in both high and low IgE responders providing an ideal opportunity to evaluate different approaches to modulating the IgE response [51].

Table 1. The number of ricin binding sites on purified splenic CD4 and CD8 positive T cells prepared by panning to 98% purity were compared using ^{125}I radiolabelled ricin. A binding site is defined as the capacity to bind one molecule of ricin.

	Ricin binding sites per cell	
	Unimmunized	Immunized
CD8 ⁺ T cells	$3.16 \times 10^6 \pm 1.76 \times 10^6$	$3.92 \times 10^7 \pm 2.21 \times 10^6$
CD4 ⁺ T cells	$2.94 \times 10^6 \pm 1.43 \times 10^6$	$3.19 \times 10^6 \pm 1.08 \times 10^6$

The effect of ricin on T-cells and cytokine production

Ricin is one of the most potent toxins ever described. It is capable of poisoning a number of mammalian cells and gains entry through cell surface glycoproteins which bind to the galactose receptor on ricin β chain. Ricin α chain is released into the cytosol and inactivates 28s RNA [52]. The ratio of CD4⁺ to CD8⁺ cells progressively increased from day 3 in parallel with the increase in serum IgE [46,53]. This is due to a decrease in the number of CD8⁺ cells rather than to an increase in the number of CD4⁺ cells. When stimulated with phytohaemagglutinin (PHA) or concanavalin A (Con A) CD8⁺ cell proliferation is nearly 100 times more susceptible to ricin than that of CD4⁺ T-cells [46,54]. Ricin alone had no effect on serum IgE or IgG levels and we have not detected IgE antibody responses to ricin. It appears that the bystander antigen is required for an IgE response to be generated and suggests that an immunological stimulus is required which makes the CD8⁺ T-cells more susceptible to ricin. Using ^{125}I radiolabelled ricin we found that the amount of ricin bound by CD8⁺ T-cells 24 h after immunization had increased over 10-fold while binding to CD4⁺ T-cells was unchanged (Table 1). However, by cytofluorographic

analysis with FITC ricin we found that not all CD8⁺ T-cells bound more ricin than CD4⁺ T-cells which may explain why not all CD8⁺ T-cells are destroyed by ricin.

To determine what effect treatment with ricin and antigen had on cytokine production, the amount of IFN γ produced by PHA stimulated CD8⁺ and CD4⁺ T-cells obtained at day 24 (Fig. 2) was compared in animals given saline, antigen and antigen + ricin. The amount of IFN γ produced by cells taken from animals which had been given antigen and ricin was 5–10-fold lower as compared with those isolated from animals given antigen or ricin or saline alone [54].

Conclusion

Ricin enhances the IgE response to bystander antigens such as ovalbumin (OVA) and bee venom phospholipase A₂ (PLA₂) in low and high IgE responder strains of rat. Repeated injection of these animals with antigen and ricin generated very high total (30–50 $\mu\text{g/ml}$) and antigen-specific (> 1000-fold) serum IgE levels which lasted for 9 months and with further booster injections could be maintained for over a year. The increase in IgE is paralleled by a decline in number of CD8⁺ but not CD4⁺ T-cells in the spleen. Increased serum IgE levels and decreased numbers of CD8⁺ T-cells were only seen when ricin was administered together with antigen, or 24 h later, suggesting that the toxin was affecting cells which were activated as a consequence of immunization. The identity of these ricin-sensitive cells was investigated in radioligand binding studies with ^{125}I ricin. There was no apparent difference in number of ricin binding sites on splenic CD4⁺ or CD8⁺ T-cells from untreated rats. However, the number of ricin binding sites on CD8⁺ T-cells from immunized rats was 10-fold higher than on comparable CD4⁺ T-cells. The effect of ricin on cytokine production was also studied. When stimulated with Con A, spleen cells from rats immunized with ricin + antigen produced substantially less IFN γ than those of control animals. Thus a sub-population of CD8⁺ T-cells appears to be activated within 24 h of immunization with antigen. Depletion of these cells with a sublethal dose of ricin results in a massive increase in serum IgE and a decreased capacity of splenic T-cells to produce IFN γ . These data suggest that CD8⁺ T-cells, activated early in the immune response, play an important part in IgE regulation and cytokine production.

Acknowledgment

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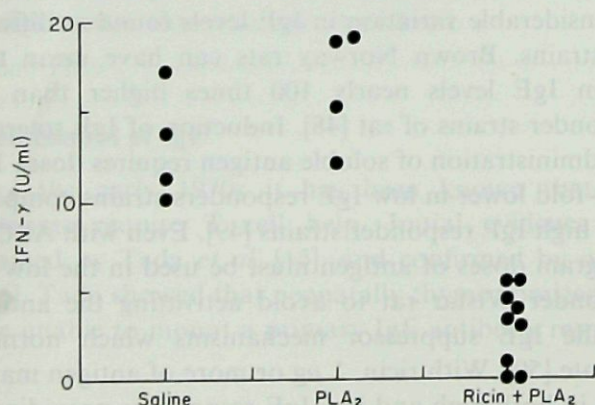


Fig. 2. IFN γ generation from PHA-stimulated Hooded Lister rat spleen cells [54].

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