

CD8 T-Lymphocyte-Mediated Regulation of Ovalbumin-Specific Murine IgE Responses

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CD8 T cells are able to reduce irrelevant antigen-specific IgE via bystander suppression once specifically activated within recipient animals.

Introduction

In recent years there has been increasing evidence to suggest that CD8 T cells, traditionally seen as cytotoxic cells, play an important role in the regulation of an IgE response [1]. Previous studies in our laboratory using a rat model of induced IgE have demonstrated that in vivo depletion of CD8 T cells greatly enhanced the ensuing IgE response, and that this enhancement could be suppressed by the adoptive transfer of antigen-specific, MHC class-I-restricted $\alpha\beta$ TcR CD8 T-cell clones back into the recipient rats [2, 3]. To define the mechanisms by which CD8 T cells suppress IgE, a murine model was set up in both high-responder BALB/c and mid-responder C57BL/6 mice. Previous work has shown both strains able to generate vigorous total and ovalbumin (OVA)-specific IgE responses to OVA/alum in vivo, without prior CD8 T-cell depletion as in the rat model, as well as a strong specific T-cell proliferative response in vitro.

In this study we demonstrate that antigen-specific in vivo primed CD8 T cells suppress both total and specific IgE responses, without affecting the concomitant IgG1 response, and that this suppression correlates to the priming dose administered to donor mice, and the number of cells adoptively transferred into recipients. We also show that

Methods

Chicken egg OVA, bovine serum albumin (BSA) and keyhole limpet haemocyanin (KLH; Sigma) was alum precipitated directly prior to intraperitoneal immunisation. BALB/c and C57BL/6 (Harlan Olac) mice were immunised on day 0, then serum samples collected via the tail vein weekly until day 28. Total IgE levels were measured by a standard sandwich ELISA using 3 μ g/ml LO-ME-3 and 3 μ g/ml biotinylated OX-20 antibody pairs (Serotec). Specific IgE was measured by a standard passive cutaneous anaphylaxis protocol.

Splenocytes from C57BL/6 mice immunised 21 days previously with 100 μ g OVA/alum were used for intraperitoneal adoptive transfer at the time of immunisation. CD8 splenocytes were obtained using Magnetic Activated Cell sorting (Miltenyi). Briefly, cells from lymphoprep spleens were incubated with anti-CD8 microbeads for 30 min, then passed through a magnetised column. When released from the magnetic field, CD8 splenocytes of >98% purity as measured by FACs analysis were eluted.

Results and Discussion

High-responder BALB/c and mid-responder C57BL/6 mice were immunised intraperitoneally with between 1 and 1,000 μ g OVA/alum. BALB/c mice made higher levels of total IgE (20 ± 1.5 μ g/ml, day 7) as compared with C57BL/6 mice (6 ± 2 μ g/ml, day 7), though more OVA-specific IgE

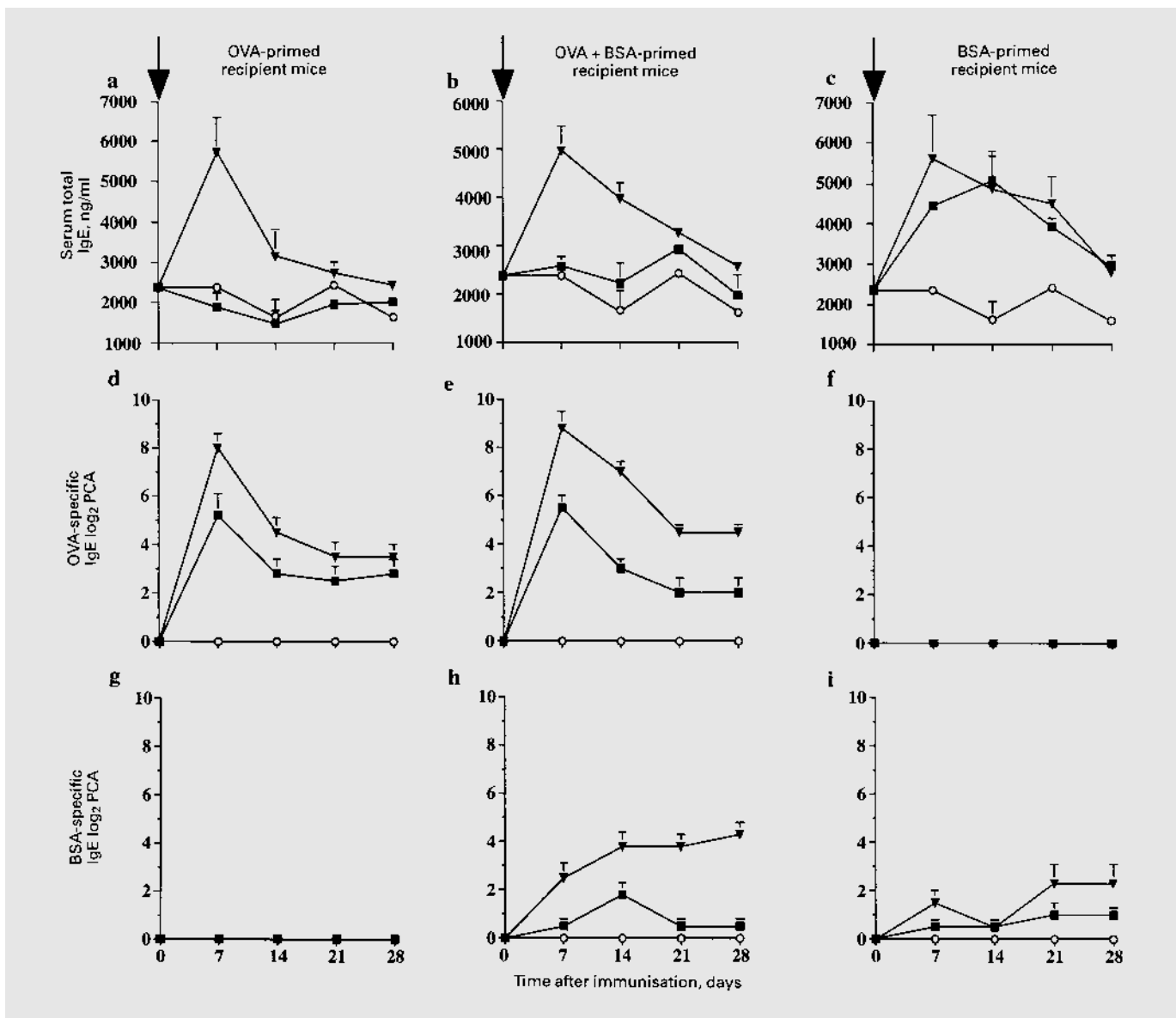


Fig. 1. C57BL/6 mice immunised with 100 µg of either OVA/alum (**a, d, g**), BSA/alum (**c, f, i**) or a combination of the two (**b, e, h**) (arrows) directly following the adoptive transfer of 1×10^6 CD8 T cells from donor mice primed 21 days previously with 100 µg OVA/alum (■) or saline (▲). Serum total IgE levels (ng/ml) are shown in **a, b** and **c**, OVA-specific IgE levels (log₂ PCA titre) are shown in **d, e** and **f**, and BSA-specific IgE levels (log₂ PCA titre) are shown in **g, h** and **i**. Background antibody levels are represented on each graph (○).

was detected in C57BL/6 mice (11 PCA titre log₂). OVA-specific IgG peaked at day 21 in both strains, increasing to 10^6 units/ml. The OVA-specific T-cell response was mapped in these animals, and was found to peak at day 21. Splenocytes at this time point from mice primed with between 1 and 1,000 µg OVA/alum were adoptively transferred into C57BL/6 mice immunised with 100 µg

OVA/alum. Total IgE levels were suppressed up to 7-fold, and OVA-specific IgE suppressed up to 16-fold at day 7 of the response, as compared to levels seen in mice receiving saline-primed cells. The degree of suppression approximately correlated to the priming dose of OVA/alum. OVA-specific IgG1 levels were unaffected by adoptive transfer, with all recipient mice generating high titre responses. To

investigate the relevant cell population and number required for optimal suppression, day-21 splenocytes from 100- μ g OVA/alum-primed mice were positively selected for CD8 cells. Between 10^3 and 10^6 CD8 T cells were then adoptively transferred with 100 μ g OVA/alum. Total IgE levels were suppressed up to 7-fold, and OVA-specific IgE suppressed up to 16-fold at day 7 of the response as compared to levels seen in mice receiving saline-primed cells. The degree of suppression closely correlated to the number of cells given. OVA-specific IgG1 levels were unaffected by adoptive transfer. To investigate whether the suppression seen was antigen-specific, recipient mice were immunised with 100 μ g of either OVA/alum, BSA/alum or a combination of the two, following the adoptive transfer of 10^6 OVA-primed CD8 T cells. Total IgE levels were suppressed up to 4-fold, and OVA-specific IgE suppressed up to 12-fold in recipient mice immunised with OVA-alum alone (fig. 1a, d). Total IgE levels were suppressed up to 3-fold, and OVA-specific IgE suppressed up to 16-fold in recipient mice immunised with OVA/alum and BSA/alum. BSA-specific IgE was suppressed by up to 12-fold in these mice (fig. 1b, e, h). Total IgE and BSA-specific IgE levels were not significantly suppressed in mice immunised with BSA/alum alone (fig. 1c, i). This experiment was repeated using KLH as the irrelevant antigen, with almost identical results.

This study clearly demonstrates the suppressive potential of CD8 T cells generated *in vivo* in response to OVA/alum. This suppression has been shown to correlate with the priming dose and cell number, and also be restricted to IgE rather than IgG. Antigen specificity has been

demonstrated, although the mechanism allows bystander suppression of irrelevant antigen-specific IgE in mice making a concomitant response to OVA. Further investigation is required to further determine the mechanism by which CD8 T cells suppress IgE. Several candidate models have been suggested. McMenamin et al. [4] have proposed a model of $\gamma\delta$ TcR CD8 T-cell suppression mediated by IFN- γ production deviating the type-2 IgE response to a type-1 response. Conflicting data have been generated by Powrie et al. [5] and Chen et al. [6] who observed $\alpha\beta$ TcR CD8 T cells suppressing IgE via a mechanism unrelated to IFN- γ production, implying the involvement of other immunosuppressive cytokines such as IL-10 and TGF β . Immune deviation by any of the afore-mentioned cytokines would explain the bystander suppression seen in our model, although suppression of OVA-specific IgG1 would also be expected. Preliminary data from our model suggests that neither IFN- γ nor IL-10 production from the CD8 T cells generated is responsible for the suppression seen, which raises the possibility of cytotoxic killing of IgE-producing B cells as a mechanism [7]. This study confirms the importance of CD8 T lymphocytes in the regulation of allergic responses and takes us closer to understanding the mechanism by which IgE can be suppressed.

Acknowledgement

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