

The IgE Antigen Receptor: A Key Regulator for the Production of IgE Antibodies

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Key Words

Gene targeting · IgE · Membrane immunoglobulins ·
Memory · Affinity maturation

Abstract

Immunoglobulins in general form a substantial component of serum proteins, and play a role in homeostatic mechanisms, a first line of defense against pathogenic organisms and in immunological memory. In the secreted form, immunoglobulins represent the effector arm of the humoral immune system. However, immunoglobulins are not only secreted, but can also be expressed on the surface of a B lymphocyte (membrane immunoglobulin), and, in this physical state, most likely convey signals to steer the B cell along its differentiation pathway. A step forward in the understanding of the role of membrane immunoglobulins other than membrane IgM or IgD was achieved with two mouse lines with mutations in the epsilon heavy chain gene. In IgE^{ΔM1M2} mice serum IgE is reduced to less than 10% of normal mice, while IgE^{KVKΔtail} mice show a reduction of 50%, reflecting a serious impairment of the IgE-mediated immune response. We think that the cytoplasmic tail of IgE is involved in a signal transduction which leads to the expression of high quantities and qualities of secreted IgE immunoglobulins.

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Introduction

Upon activation by antigen, B cells follow one of two differentiation pathways. First, as predicted by the clonal selection theory, cross-linking the B cell receptor with antigen can lead to clonal expansion and differentiation directly into plasma cells, which are basically antibody-secreting factories. Second, they may give rise to germinal centers [1–4], specialized structures within lymphoid organs, where successive rounds of mutation of the immunoglobulin variable region genes are followed by expression of the gene products on the cell surface. Surface expression is followed by selection of the cells on the basis of the affinity of these mutated immunoglobulins [5–7]. The membrane-bound immunoglobulins are critical in the generation of B cell-mediated immune responses. Throughout the differentiation process, the surface-bound immunoglobulin must perform two functions: signal transduction and the transport of captured antigen to an endosomal compartment for processing and presentation on the cell surface in association with the major histocompatibility complex class II. The question of how the cytoplasmic domains of immunoglobulin might contribute to immune responses and B cell memory was addressed by Achatz et al. [8] and Kaisho et al. [9]. The cytoplasmic domain of IgE and IgG1 was deleted for the conserved three charged amino acids KVK (lysine-valine-

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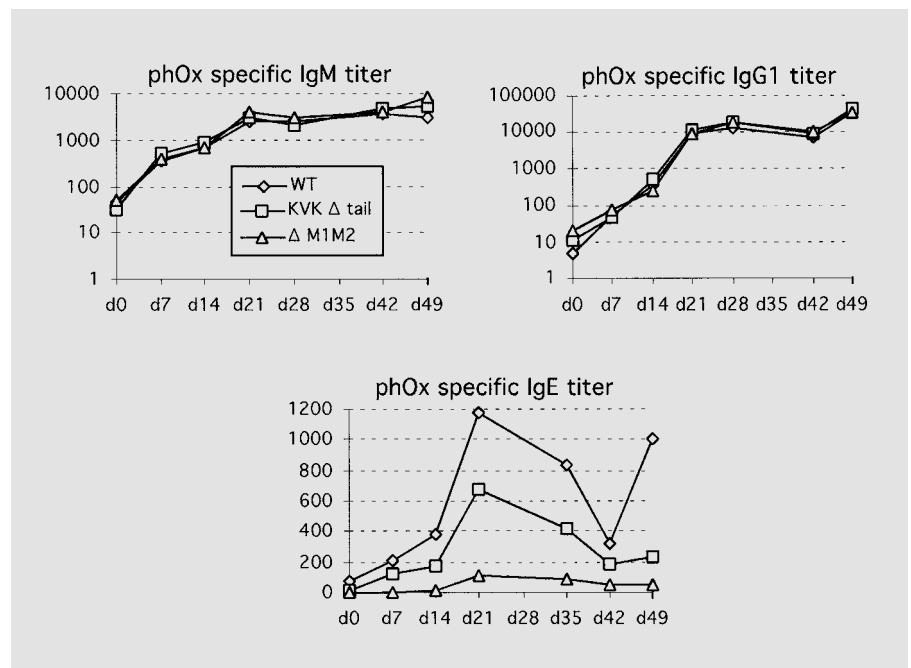


Fig. 1. PhOx-based immunization protocol: isotype-specific phOx titers. Mice were immunized and bled at the indicated time points. IgM and IgG1 responses showed no differences between wild-type (WT) and mutant strains. In IgE^{KVKΔtail} and IgE^{ΔM1M2} mice the IgE response was dramatically impaired.

lysine), which function as a membrane anchor. In the case of membrane-bound μ -heavy chain, these charged membrane anchor residues are essential to keep the protein in the membrane, but they appear to contribute little to signal transduction [10]. IgE^{KVKΔtail} as well as IgG1^{Δtail} showed a dramatic reduction of the quantitative and qualitative primary and secondary immune response. Thus, the cytoplasmic tails of the ϵ - and γ 1-heavy chains are essential for normal immune responses, regarding expansion and maintenance of mIgE- and mIgG1-bearing B cells, which are additionally the prerequisite for the expression of secreted immunoglobulins. To study the specific function of the cytoplasmic tail of mIgE, regarding the quantity and quality of the immune response, we investigated a 2-phenyloxazol-5-one (phOx)-based immunization protocol [11] (fig. 1).

Materials and Methods

Immunization

Mice were primarily immunized subcutaneously with 20 μ g phOx11-OVA, precipitated in alum with *Bordetella pertussis* as adjuvants. At day 14 and 42, the mice received booster immunizations with 20 μ g phOx11-OVA s.c. and 20 μ g phOx11-OVA i.p.

ELISA Assays

Serum was taken at different times (day 0, 7, 14, 21, 28, 42 and 49) after immunization. For determining the titer of the IgM, IgG1

and IgE responses, ELISA plates were coated with phOx₃₅-BSA. Serially diluted serum samples were added. Sera (5 animals/group) were pooled and specific IgM, IgG1 and IgE were detected with alkaline phosphatase-conjugated isotype-specific goat α -mouse antibodies. Results are expressed as the serum dilution where half-maximum absorbance was obtained for IgM, IgG1 and IgE.

Spot-ELISA

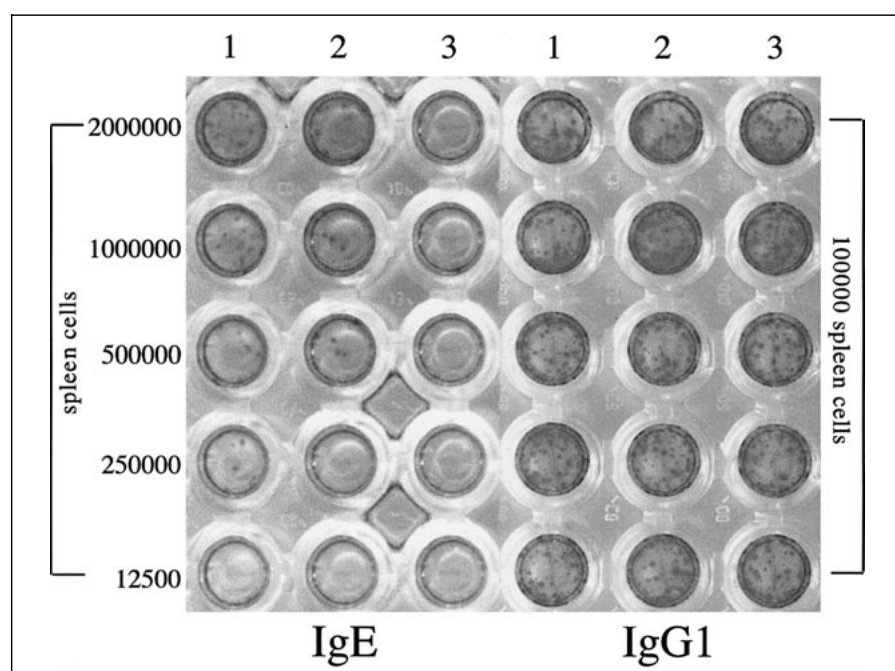
Microtiter plates were coated with rat monoclonal antibody (mAb) 84-1C to IgE and goat mAb to IgG1. After overnight incubation, wells were blocked with 1% bovine serum albumin. Serial dilutions of spleen cells after isotonic lysis were cultured for 18 h in complete Iscove's medium. Plates were washed and incubated with alkaline phosphatase-conjugated rat mAb EM95.3 to IgE and alkaline phosphatase-conjugated rat mAb to IgG1. Spots were developed with the substrate 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) in 0.6% low-melting agarose [12].

Results

phOx-Specific Titers of IgM, IgG1 and IgE Antibodies

In a first approach, IgM, IgG1 and IgE antibody titers (fig. 1) were measured following immunization with the thymus-dependent hapten phOx coupled to ovalbumin. Serum levels of specific IgM and IgG1 were comparable in wild-type and mutant mice. After primary immunization an increase of 10^2 in phOx-specific IgM antibodies could be detected. After the booster immunization only a slight increase of IgM-specific antibodies was measured.

Fig. 2. Spot-ELISA. In IgE Δ M1M2 mice (lane 3) IgE-secreting plasma cells are missing. In IgE^{KVK Δ tail} mice (lane 2), the number of IgE-secreting plasma cells is 50% decreased if compared to wild-type mice (lane 1). The IgG1 control showed no differences between wild-type (lane 1) and mutant strains (lanes 2, 3).



In the following weeks the titer remained relatively stable and a weak memory response was measured after the second booster immunization. When we investigated the phOx-specific IgG1 titer we could describe an immunization pattern typical for isotypes of the secondary and tertiary immune response. A rapid increase in titer from day 7 to day 21 was detected. The second booster immunization gave rise to antibodies of the memory response with an additional quantitative increase in phOx-specific IgG1 titer. In contrast, dramatic differences in serum IgE levels were found in wild-type and mutant mice. In wild-type mice, a clear secondary response was seen after the first booster immunization and even a clear memory response could be described. In KVK Δ tail mice the secondary immune response was decreased and the memory response was absent. In Δ M1M2, phOx-specific IgE antibody titers remained at detection levels.

IgE- and IgG1-Secreting Plasma Cells in Wild-Type, IgE^{KVK Δ tail} and IgE Δ M1M2 Mice

To determine whether the reduction in IgE titers in the mutant strains was caused by reduced levels of IgE production per cell or by a smaller number of cells that produce IgE, we measured the number of IgE-secreting cells at day 21 of our immunization protocol (fig. 2). In wild-type mice about 20 IgE-secreting plasma cells per $2 \cdot 10^6$ spleen cells could be detected. In IgE^{KVK Δ tail} mice 9 IgE-

secreting plasma cells per $2 \cdot 10^6$ spleen cells were found and in IgE Δ M1M2 mice IgE-secreting plasma cells were missing. This shows very clearly that the defect in the mutant mice strains lies in the recruitment of IgE-secreting plasma cells and underlines the importance of the cytoplasmic tail of the IgE antigen receptor.

Discussion

A step forward in the understanding of the role of membrane Igs was achieved with the two mouse lines IgE^{KVK Δ tail} and IgE Δ M1M2. In IgE Δ M1M2 mice serum IgE is reduced to less than 10% of normal mice, while IgE^{KVK Δ tail} mice show a reduction of 50%, reflecting a serious impairment of the IgE-mediated immune response. These results imply that the reduced IgE titers found in both mutant lines are solely a reflection of the loss of biological activities associated with the transmembrane and cytoplasmic domains of IgE. We, therefore, assessed the immune response for the mutant mice. First, mice were immunized with the T cell-dependent antigen phOx and specific antibody titers were measured. Serum levels of specific IgM and IgG1 were comparable in wild-type and mutant mice. In contrast, in IgE Δ M1M2, phOx-specific IgE antibodies could barely be measured. In IgE^{KVK Δ tail} mice titers were 50–80% lower than in control mice. In

IgE^{EKVKΔtail} mice a clear secondary response, as characterized by a strong and fast rise in specific antibody titer, was absent. This may additionally be interpreted as a qualitatively changed immune response. Second, with the help of an IgE-specific spot-ELISA, we found that the reduced IgE levels in the mutant mice are reflected in a lower number of IgE-secreting cells, indicating that a lower number of IgE-producing cells is effectively recruited in the immune response. Our data clearly show that the transmembrane domain of IgE is indispensable for a T cell-dependent IgE-mediated immune response, and that the cytoplasmic tail not only determines the absolute amount of IgE produced, but also the quality of the antibodies. How does this work? Two hypotheses can be brought forward. The first implies that signals generated via membrane-bound Ig are needed at all times, not only for the maturation process, but also for the expansion of antigen-specific cells. The second hypothesis postulates that antigen presentation to T helper cells is necessary at all times during an antibody response and that the antigen receptor is the only device for an effective antigen presentation. If the membrane domains are a prerequisite to recruit cells for

the production of secreted IgE, how can we explain that residual serum IgE is present in IgE^{ΔM1M2} mice? A possible explanation may be that serum IgE can also be expressed over the bypass of the IgG1 antigen receptor. Yet, the poor serum IgE in IgE^{ΔM1M2} mice argues against the notion that consecutive switch recombination via γ 1 is a major operational mechanism in the generation of IgE-expressing B cells in the mouse. At the time it is not clear which function the antigen receptor performs: interactions with external ligands, maintenance of a tonic signal generated by the mere presence of the receptor in the membrane, or capture of antigen.

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