Ig-specific T Cell Receptor-transgenic T Cells Are Not Deleted in the Thymus and Are Functional In Vivo

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Summary

The mechanisms that induce T cell tolerance to circulating self-proteins are still controversial, and both the deletion and selection of autoreactive T cells have been observed in the thymus of transgenic mouse models. To address the question of the induction of tolerance to circulating self-constituents, a T cell receptor-transgenic mouse specific for the serum protein immunoglobulin (Ig) γ and (IgG2α) was generated. The choice of an allotype-specific T cell also allowed the generation of transgenic control mice not expressing the self-antigen. It was found that the transgenic T cells were not deleted in the thymus, did not become tolerant in the periphery, and regulated the function of γ2α-positive B cells as shown by the lack of IgG2α protein in the serum of the transgenic mice. In spite of this activity in vivo, the transgenic T cells did not proliferate in vitro in response to the allotype-specific peptide. Interestingly, antigen-specific T cell proliferation could be restored if the transgenic mice were previously challenged to induce IgG2αβ responses. After this challenge, IgG2αβ protein in the serum of the transgenic mice could be partially restored, although still remaining much lower than in control mice. In addition, there was a dramatic increase in serum IgE levels, suggesting that newly generated γ2αβ-secreting B cells can be induced to switch to IgE in the presence of allotype-specific T cells. These results indicate that Ig-specific T cells may represent a late-acting form of T cell help for the regulation of the IgG2α-to-IgE class switch.
whether anti-Ig T cells are associated with autoimmune diseases, such as rheumatoid arthritis.

The possibility that circulating Ig-specific autoreactive T cells may be harmless for the organism should be considered. In line with the hypothesis that B cells cannot prime virgin T cells (16), it may be thought that most antiisotype IgG2aβ protein might develop tolerance to B cells presenting epitopes of their own Ig (17). However, it has also been shown that, if correctly activated by CD4+ helper T lymphocytes, B cells may acquire all of the costimulatory signals necessary to prime virgin T cells (18). Furthermore, it has been recently demonstrated that, if activated via the B cell receptor, B cells transduce all of the signals necessary for virgin T cell proliferation and lymphokine production (19).

To study the fate of T cells specific for Ig as circulating self-constituents, we have generated a TCR-transgenic mouse model specific for the serum protein IgG2aβ. There were two reasons for the choice of this model: first, an allotype-specific T cell would allow us to generate transgenic control mice that do not express the self-antigen; second, in the absence of tolerance induction, the fate of B cells after the interaction with allotype-specific T cells could be followed.

In the mouse model described in this study, the transgenic T cells are not deleted in the thymus, do not become tolerant in the periphery, and regulate the function of γ2aβ-positive B cells, as shown by the lack of IgG2aβ protein in the serum of the transgenic mice.

Interestingly, when the animals’ immune system was challenged by immunization, the IgG2aβ protein in the serum remained much lower than in controls, and a dramatic increase in IgE was observed. This suggests that the γ2aβ-secreting B cells can be induced to switch to IgE in the presence of allotype-specific T cells.

### Materials and Methods

**Mice.**  BALB/c, DBA/2, and CD1 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and bred in our conventional animal facility. CD17 mice were purchased from the Jackson Laboratory (Bar Harbor, ME).

**Construction of the α Transgene.** The α and β chains of the TCR were both cloned from the B5 T cell clone. The α-rearranged coding cDNA (1,300 bp) was amplified by anchored PCR as described (20), cloned in the XhoI-Smal restriction sites of pGEM7zf (Promega Corp., Madison, WI), and sequenced. The oligonucleotides used were 5'GTTTRANSGENICTCTTC-AGGAATTTTTTTCTTGACCY (used in 3') and 5'TACTCGAGTCGACATCGATTTTTTTTTTTTY and 5'TACTCGAGTCGACATCGA- 

**Construction of the β Transgene.** A 900-bp XhoI-Smal fragment containing rearranged Vβ14/Dβ1.1/Jβ2.5/Cβ2 sequences was generated by reverse transcription (RT) PCR, cloned into pGEM7zf, and sequenced. The oligonucleotides used for the amplification were 5'GGTCGACTCGAGAAAGACCATCTT-GAACTATY (used in 5') and 5'GGTCGACTCGAGAAAGACCATCTT-GAACTATY (used in 5'). The RT-PCR amplification cycle was performed at the following conditions: 60 s at 94°C, 60 s at 60°C, 60 s at 72°C, for 30 cycles and 1 cycle at 72°C for 10 min to terminate the reaction. The SalI-BamHI fragment was inserted into the pHSE3'-transgenic vector. The two XhoI-XhoI fragments, from pHSE3'α and pHSE3'β, corresponding to the α and β chains, were used to produce transgenic mice.

**Generation of Transgenic Mice.** Transgenic mice were generated as described by Hogan et al. (22). 

**Flow Cytometry.** Single-cell suspensions of 10⁶ thymocytes or lymph node cells were pelleted and resuspended in 50 µl of PBS with 5% FCS and 0.05% sodium azide with the appropriate amounts of antibodies and incubated for 30 min on ice. Cells were then washed once with 1 ml of PBS. Second-reagent incubation was performed in 50 µl of streptavidin–PerCP (Becton Dickinson & Co., Mountain View, CA) conjugated at the concentration indicated by the manufacturer, for 15 min on ice in the dark. The first-step antibodies were CD4, FITC-coupled anti-mouse CD4 (20 µg/ml; PharMingen, San Diego, CA); CD8, PE-coupled anti-mouse LyT-2 (20 µg/ml; PharMingen); Vβ14, biotinylated anti-mouse Vβ14 (20 µg/ml; PharMingen). Three-color analysis was performed on 2 × 10⁶ viable cells with a FACScan® (Becton Dickinson & Co.); the plates were plotted on a quadruple logarithmic scale.

**T Cell Proliferation Assay.** The cells were isolated from lymph nodes and cultured in 96-well plates for 4 d in IMDM (Sigma Chemical Co., St. Louis, MO) supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (all from Sigma Chemical Co.) and 5% heat-inactivated FCS (Boehringer Mannheim Corp., Indianapolis, IN), with serial dilution of synthetic peptide. The cells were incubated in triplicate wells, and 1 µCi of [³²P]thymidine was added to the wells during the last 16 h of culture. The plates were then harvested and counts per minute were determined by means of liquid scintillation counting.

**Measurement of Ig Isotypes and Cytokines.** Serum Ig levels and cytokine amount were determined by solid-phase ELISA with the use of a sandwich assay. High-bind plates (Costar Corp., Cambridge, MA) were coated with different antiisotype and anticytokine antibodies at 10 µg/ml in PBS and 50 µl/well. After incubation for 24 h at 4°C, the wells were washed five times with tap water (for isotype ELISA) or PBS, 0.05% Tween 20 (for cytokine measurement). The plates were blocked with 200 µl/well of PBS, 0.5% BSA for 1 h at 37°C, and then rinsed five times with tap water (for isotype ELISA) or PBS, 0.05% Tween 20 (for cytokine measurement). Mouse Ig or cytokine standards (50 µl/well) at various dilutions starting from 10 µg/ml in PBS, 0.5% BSA and serum samples or culture supernatants were loaded in the wells and incubated for 24 h at 4°C. After washing the wells with PBS–Tween or water, 50 µl of antiisotype or anticytokine biotin-coupled secondary antibodies were added for another 1-h incubation at 37°C. The wells were then washed six times and incubated 30 min with 100 µl of alkaline phosphatase–conjugated streptavidin (Boehringer Mannheim Corp.) at the manufacturer’s recommended concentration. The developing substrate (0.1% p-nitrophenyl phosphate) was added for at least 30 min at room temperature, and the plates were read with a reader (Hewlett Packard Co., Palo Alto, CA). IFN-γ and IL-4 were used as standard
curves were from Genzyme Corp. (Cambridge, MA). IgG1 serum level was measured by inhibition ELISA. After overnight coating with the N1G9 (IgG1) antibody and a 1-h blocking in PBS, 0.5% BSA, 0.05% Tween 20, the plates were incubated for 1 h at 37°C with 50 μl of a mixture of 50% sera and 50% biotin-conjugated goat anti-mouse IgG1 antibody (Southern Biotechnology Associates, Birmingham, AL) at a final concentration of 0.05 μg/ml. After PBS, 0.05% Tween 20 washing, the plates were treated, as previously described, with alkaline phosphatase-conjugated streptavidin and revealing substrate.

**Antibodies.** The isotype-specific antibodies were as follows: anti-mouse IgE, 95.3 and biotin-conjugated anti-mouse IgE (PharMingen); anti-mouse IgM, R33-42-12 and biotin-conjugated R 33-60; anti-mouse IgG1, biotin-conjugated anti-mouse IgG1 (Southern Biotechnology Associates); anti-mouse IgG2b, R 14-50 and biotin-conjugated goat anti-mouse IgG2b (Southern Biotechnology Associates; anti-mouse IgG3, 2E.6 and biotin-conjugated anti-IgG2a allotypes (all from PharMingen); anti-IgE, purified anti-total IgE and alkaline phosphatase-conjugated anti-IgE (all from PharMingen). All of the antibodies were kindly provided by K. Rajewsky (Cologne University, Cologne, Germany). Purified and biotin-conjugated anti-IFN-γ and anti-IL-4 antibodies were purchased from PharMingen, whereas the following antibodies used for the standard curves were provided by K. Rajewsky: IgG1, N1G9; IgE, 12.2; IgG2b, D3-13 F1; IgG2a*, 42.1; IgG2a, S43.10; IgM, B1-8; IgG3, S24/63/63.

**Peptide-induced Thymic Deletion.** The peptide, not soluble in PBS, was resuspended in water at a concentration of 10 mg/ml. Mice were injected i.v. with 100 μl of peptide solution or 100 μl of water as a control.

**Propidium Iodide Analysis.** 10^6 thymocytes were incubated 30 min on ice in 1 ml of 50 μg/ml propidium iodide, 0.1% sodium citrate, 0.1% Triton X-100. Cells were then analyzed by FACS® as described (23).

**Immunolocalization of the Apoptotic Thymocytes.** Fragments of thymuses were frozen in dry ice powder and stored at −80°C. 5-μm frozen sections were cut and mounted on gelatine-coated glass slides. The cryosections were dried at room temperature for at least 1 h and fixed in acetone for 5 min. The terminal deoxyribonucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) method was carried out using the ApopTag™ kit (Oncor Inc., Gaithersburg, MD). Briefly, the cryosections were incubated at 37°C with terminal transferase enzyme and digoxigenin-dUTP. The apoptotic cells were then detected by staining with 20 μg/ml of anti-digoxigenin-rhodamine antibody Fab fragments (Boehringer Mannheim Corp.).

**Immunization Procedure and Anti-CD8, Anti CD4 Treatment.** LT2 MC1 galE mutant of Salmonella typhimurium strain (24) was

![Figure 1](image_url)

**Figure 1.** Thymus T cell population in TCR transgenic mice. Thymocytes from 4–6-wk-old α/β Tg+b−, Tg+b−–transgenic mice were stained with antibodies specific for CD4 (horizontal axis) and CD8 (vertical axis, upper panel) and Vβ14 variable chain (lower panel). Nontransgenic and α/β-transgenic thymocytes were compared. The lower panel also shows the analysis of Vβ14 expression in the four thymic subpopulations: CD4+CD8− (CD4), CD4+CD8+ (DP), CD4−CD8+ (CD8), and CD4−CD8− (DN).
grown overnight in oxoid brain-heart (Sigma Chemical Co.) infusion (37 g/liter of distilled water) at 37°C. Bacteria were washed three times with 0.1 M sodium bicarbonate, pH 8.4, and injected intraperitoneally into the mice. The first immunization was performed with \(2.5 \times 10^6\) cells in 300 µl and the second one with \(10^9\) LT2 M1C in 500 µl of sodium bicarbonate. The 3.168 anti-CD8 (25) and the GK1.5 anti-CD4 (26) mAbs were purified from supernatants by protein G affinity chromatography (Pharmacia Biotech Inc., Piscataway, NJ) according to recommended procedures. 500 µg of anti-CD8 and anti-CD4 antibodies were injected intraperitoneally into mice on days 0, 1, and 2.

**Thymectomy and ELISPOT Assay.** Thymectomy and ELISPOT assay were performed according to Sjokin et al. (27) and Czerkinsky et al. (28), respectively.

**Results**

**Selection of the T Cell Clone for Constructing Transgenic Mice.** The B5 clone was generated by Bartnes and Hannestad (29) by the injection of a γ2aβ mAb into BALB/c mice. The T cell is a CD4\(^+\), Th1, I-A\(^d\)-restricted clone, and the epitope recognized by B5 TCR was mapped in the CH3 domain of IgG2aβ (residues 435–451) (30). We selected B5 because it has been shown to suppress the IgH-1b allotype in vivo (29); moreover, the choice of an allotype-specific T cell allowed us to generate transgenic control mice that do not express the self-antigen.

The rearranged α and β chains of the TCR were cloned by anchored PCR (20) or RT-PCR, respectively, sequenced, and then introduced into the pHSE3' transgenic vector (21). Transgenic mice were generated using the two XhoI–XhoI fragments corresponding to the functionally rearranged TCR genes containing V\(\alpha\)4DA33 (31) and V\(\beta\)14 (32) variable chains (data not shown). Separate α and β chain–transgenic mice were established by injecting (DBA/2 × BALB/c)F2 fertilized eggs, and the two lines were then bred together to produce mice expressing the complete TCR. Transgenic mice were generated on two different genetic backgrounds as described in Materials and

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**Figure 2.** Transgenic thymocyte deletion after peptide injection. Immunofluorescent staining with the TUNEL method of Tg\(^+\)β\(^-\) transgenic mice thymic cryosections: (A) noninjected, (B) injected with 100 µl of water, and (C) injected with water-resuspended (100 µl) peptide, compared with (D) peptide-injected nontransgenic mice. (E) CD4 (horizontal axis) and CD8 (vertical axis) staining of Tg\(^+\)β\(^-\) mice thymus compared with (F) nontransgenic mice after peptide injection. The total thymus cell number recovered from each mouse was between 80 and 100 million. Bar, 500 µm.
Methods: Igh-1α/α (Tg+b- not expressing the self-antigen) and Igh-1β/β (Tg+b+, expressing the self-antigen).

Thymic T Cell Populations in TCR-Transgenic Mice. Vβ14-expressing T cells could be followed by the 14.2 mAb (33); anti-Vβ14 staining showed that thymic Vβ transgene expression was comparable to the pattern of TCR expression in normal animals. Three different populations were observed in the thymus: high (CD4 or CD8 single-positive), intermediate (double-positive) and low (double-negative) Vβ14-expressing T cells (Fig. 1). No thymic deletion of the transgenic T cells was found as CD4, CD8, and Vβ14 triple staining showed a slight increase in the CD4+CD8- cells of both Tg+b- and Tg+b+ mice, compared with their nontransgenic littermates (Fig. 1).

Peptide-induced Apoptosis of Double-positive T Cells. To assess whether transgenic thymocytes were susceptible to deletion as a mechanism of tolerance induction, we compared the thymuses of the Tg+b+ mice for apoptosis after...
injection with 1 mg/100 μl of the specific peptide (30) or water, and noninjected mice (the peptide, not soluble in saline, was dissolved in water). 10 h after injection, propidium iodide FACS analysis (23) showed <1% of apoptotic cells in both the noninjected and water-injected mice, whereas 10% of the total thymus cell population was apoptotic in those injected with peptide (data not shown). These data were confirmed by immunofluorescent staining of thymus cryosections (Fig. 2 C) using the TUNEL method (34). On sections, the preferential staining of the cortex is consistent with a deletion of double-positive T cells. An increase in the CD4 and CD8 double-negative subpopulation was detected in peptide-injected mice by means of CD4/CD8 immunofluorescence and FACS analysis (Fig. 2 E), indicating that, if deletion had occurred in the thymus of non-peptide-injected Tg+b+ mice, it would have also been detected. We therefore concluded that the transgenic T cells are not deleted in the thymus but released into the periphery.

Lymph Node T Cell Populations in TCR-transgenic Mice. Lymph node single CD4+ and CD8+ subpopulations were similar in the Tg+b-, Tg+b+, and control mice. The Vβ14 transgene was expressed in 70–80% of CD4+CD8+ cells and in 50–70% of CD8+CD4- lymphocytes (Fig. 3 A). To determine whether the T cells in the transgenic mice were functional, we measured the capacity of lymph node-transgenic T cells to proliferate in vitro to the specific peptide. Lymph node Tg+b- T cells proliferated, whereas Tg+b+ cells did not (Fig. 3 B).

Transgenic T Cells Are Functional In Vivo. To test whether this nonresponsive status of Tg+b+ transgenic T cells observed in vitro was physiologically important, self-antigen serum levels were measured. Surprisingly, IgG2a b serum levels (Fig. 4 A) were undetectable with ELISA (sensitivity 3 ng/ml), whereas IgG2a b levels were comparable with those of nontransgenic mice (Fig. 4 B); furthermore, γ2a b-secreting cells from lymph node and spleen were consistently undetectable as estimated by the ELISPOT assay, whereas 60 spot-forming cells/106 cells were measured in normal animals (data not shown). These results clearly indicate that transgenic T cells do not become tolerant in the periphery by clonal deletion or by induction of anergy but rather regulate the number of γ2a b-producing cells.

As transgenic animals have a relevant CD8+CD4+ population, a possible involvement of cytotoxic T cells in determining the observed phenotype was tested by injecting an anti-CD8-depleting mAb into mice (25): during the 7 d in which the CD8+ cells were not detectable in the peripheral blood, no increase in IgG2a b serum levels was observed (Fig. 4 C), thus suggesting that CD8+ cells do not play any major role. In contrast, treatment with anti-CD4 mAb restored IgG2a b control levels 2 d after injection (data not shown).

Transgenic T Cells Are Functional in Thymectomized Tg+b+ Mice. Adult transgenic mice were thymectomized to investigate whether only newly generated transgenic lymphocytes were involved in determining the phenotype. If this were the case, then IgG2a b control levels would have been restored once newly matured transgenic lymphocytes had been excluded. However, 5 mo after thymectomy, no increase in γ2a b serum levels was observed (data not shown), suggesting that memory is preserved.

Altogether, these data indicate that transgenic T cells may be in an undefined functional state in vivo that is not measured in vitro by the antigen-specific proliferation assay.

Transgenic T Cells Are Involved in an IgG2a b-to-IgE Class Switch. On days 0 and 7, transgenic mice were immunized with S. typhimurium to challenge the immune system and increase IgG2a b serum levels. 3 wk after immunization, a peak level of 10 µg/ml of γ2a b protein was reached, which was almost 60 times lower than that reached in the control mice (Fig. 5, A and B). Interestingly, as the other isotypes and the IgG2a b were unaffected (Fig. 6), IgE serum levels were five times higher than in the nontransgenic animals (Fig. 7 A). 4 wk after immunization, a decrease in

Figure 4. Serum levels of IgG2a b in Tg+b+ mice. (A) IgG2a b and (B) IgG2a b serum levels in 4–6 wk-old mice were measured using solid phase ELISA. (C) Animals were treated on days 0, 1, and 2 with 500 µg of anti-CD8-depleting antibody. Serum levels of IgG2a b in nontreated, PBS-treated, and antibody-treated mice were measured by ELISA.

Figure 5. IgG2a b serum levels during an immune response. Transgenic mice were immunized on days 0 and 7 with S. typhimurium (arrows). (A) Nontransgenic and (B) transgenic mice IgG2a b total serum levels, followed from 0–9 wk during immune response. IgG2a b serum levels remained 60 times lower in transgenic compared with control mice.
IgG2a\(^b\) was associated with an increase in IgE levels. The increased IgE in the transgenic (Tg\(^b\)\(^+\)) (Igh-1\(^b\)) were all of the b allotype (Fig. 7 B), suggesting that they derive from IgG2a\(^b\)-positive cells. A similar response was also observed with immunization of the transgenic mice with nonmicrobial protein antigens (data not shown).

The State of Transgenic T Cell Responsiveness Is Changed after Immunization. The immunization of the transgenic mice with *S. typhimurium* gave rise to another important finding aside from the increase in IgE serum levels. Lymph node cells from *Salmonella*-immunized transgenic mice could proliferate in vitro in response to the specific peptide (Fig. 8 A) and produce IFN-\(\gamma\) (Fig. 8 B). As late as 13 wk after immunization, the transgenic T cells still retained their different activation state, as shown by the capacity of the transgenic T cells to proliferate and produce lymphokines in vitro.

Discussion

As the mechanisms of tolerance induction to circulating self constituents are still not clearly defined, we generated a transgenic mouse model with a TCR specific for the serum protein IgG2a\(^b\). The original T cell clone from which the TCR-\(\alpha\) and -\(\beta\) chains were derived was chosen because it
mocyte selection is related to the number of TCRs engaged with peptide–MHC complexes, with negative selection occurring when this number is high (high avidity) and positive selection occurring when this number is low (low avidity) (36). As Igs are not easily processed and presented (37), we can assume that the low avidity necessary for positive selection is attributed to the low density of peptide–MHC molecules on the surface of selecting cells. We cannot exclude that the transgenic T cells express different densities of the transgenic TCR because of expression in some cells of a second TCR using the transgenic β chain and a rearranged endogenous α chain (38). High density cells would have the highest avidity for the target and might have been deleted in the thymus, leaving only low avidity cells emigrating in the periphery. Alternatively, only a particular type of cell may be involved in Ig presentation and positive selection. If, as proposed in the literature (39), B cells are the principal Ig APC in the thymus, their number might be too low to induce negative selection.

Injection of specific peptide was found to lead to double-positive cell deletion, which may be explained by either an increase in peptide density in the thymus or the involvement of APC different from the selecting cells normally used.

Tg+b+ mice have very low or undetectable IgG2aβ serum levels, and γ2aβ secreting cells are not detectable in either lymph nodes or spleen. This indicates that transgenic T cells do not become tolerant in the periphery by means of clonal deletion or the induction of anergy, but instead regulate the number of γ2aα cells. Because the β allotype of IgG2a appears to be easily suppressed (40, 41), it might be thought that the lack of tolerance of anti-β allotype-specific T cells could be a special case. Nevertheless, it has been shown that in mice transgenic for an Ig anti-IgG2a of the α allotype, the transgenic B cells do not become tolerant even at 50 μg/ml of IgG2aα (42). Thus, this feature is not restricted to the β allotype and seems to be a common characteristic of anti-Ig-specific lymphocytes.

The activity of Ig-specific transgenic T cells could not be investigated in vitro by means of the proliferation function or lymphokine secretion because these cells did not respond to the specific peptide; surprisingly, the transgenic T cells did proliferate and produce IFN-γ after immunization with Salmonella. The presentation of self-Ig determinants has been investigated by Rudensky and Yurin (37) and Bikoff and Eckhardt (43); their results suggest that B cells are the major cell type presenting Ig because dendritic cells and macrophages are inefficient. We thus assume that, before immunization, it is the γ2aβ-positive cells that mainly present the CH3 domain of their Ig, and this determinant may not be available for other APC. After interaction with B cells, the T cells were not anergized in vivo, though they were not able to proliferate in vitro.

T cell activity was confirmed in vivo by the analysis of thymectomized mice: if the T cells had been deleted or anergized, γ2aβ serum levels would have increased given the absence of newly differentiated transgenic T cells, but this was not the case. The T cells remained functional for at
Figure 9. Fate of B cells during the immune response. Antigen-specific T cells interact with IgM+ B cells that induce their maturation and class switch. After this process, the IgM+ B cells become IgG2ab-expressing cells that can present epitopes of γ2aβ and interact with allotype-specific transgenic T cells. A further class switch of the B cell is induced by this interaction. It cannot be excluded that this process induces B cell death. A possible involvement of non-B APC must be considered.

At least 5 mo after thymectomy, thus indicating that T cell memory was preserved.

After immunization with Salmonella, the increase in IgG2ab serum levels made it available for other APC that could now present the IgG2ab epitope to the transgenic T cells. The signal given by non-B APC could drive the T cells to a different functional state, which could then be investigated in vitro by peptide-specific proliferation and lymphokine secretion. Once the T cells reached this state of maturation, they preserved it for at least 13 wk.

The fate of the B cells in immunized transgenic mice was also followed during the immune response; the γ2aβ Igs reached a peak of 10 µg/ml 3 wk after immunization and started decreasing at 4 wk. At the same time, the level of IgE increased until it was five times higher than that in the nontransgenic animals. Interestingly, this increase is ascribable to IgE of the β allotype, indicating that IgEβ-positive B cells might derive from IgG2ab-positive cells. To explain this observation, we propose that during the immune challenge, when IgM-expressing B cells become IgG2ab producers, epitopes of the γ2aβ protein are presented by B cells to antiallotype transgenic T cells (Fig. 9). It can be hypothesized that the outcome of this interaction is the induction of a further switch to IgE; this is consistent with the genomic organization of the Ig locus, the γ gene being downstream to the ζ2a gene. However, at this point, we cannot distinguish isotype switching of activated B cells from differentiation (and secretion) of previously generated memory cells. In addition, since it has not been proven that memory B cells cannot switch to others isotypes, this might be the system to investigate this problem. Nevertheless, the possibility that B cells die after antiallotype transgenic T cell interaction cannot be formally excluded.

Given that transgenic T cells are not deleted in the thymus and are functional in the periphery, all of these findings indicate that Ig-specific T cells could exist in normal individuals and may represent a late-acting form of T cell help for the regulation of the IgG2a-to-IgE class switch.

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