IgE CLASS-RESTRICTED TOLERANCE INDUCED BY NEONATAL ADMINISTRATION OF SOLUBLE OR CELL-BOUND IgE

Cellular Mechanisms

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Unraveling the pathophysiology of IgE-mediated allergic disorders requires a thorough understanding of the complex mechanisms that control IgE antibody synthesis. The selective activation and further maturation of the IgE B cell are regulated by both antigen-specific and non-antigen-specific, but isotype-restricted, T cells and the soluble factors that they produce (1–6). Although the manner by which antigen-specific and isotype-restricted regulatory events are coordinated is still unresolved, studies of Ishizaka and colleagues (7–13) and of our group (14–19) indicate that the Fc determinants of IgE and the correspondingly specific Fc receptors for IgE (FcRϵ)[1] on lymphocytes contribute significantly to such regulatory mechanisms.

IgE antibody responses can be significantly enhanced in experimental animals in which sensitization with antigen has been properly correlated with nonspecific perturbations such as low dose irradiation or treatment with immunosuppressive drugs (13, 20–23). Similar perturbations, perhaps resulting from common respiratory virus infections (15), could be responsible for development of high IgE responses in individuals exposed to allergens over a sustained period of time (21), and are probably involved in the enhanced IgE production that accompanies parasite infections (22). Certain of the experimentally induced perturbations in normal mechanisms controlling IgE antibody responses can be selectively reversed by in vivo administration of biologically active soluble factors, obtained from body fluids of living animals (23).

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[1] *Abbreviations used in this paper:* B lymphocyte, bone marrow–derived lymphocyte; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; DTT, dithiothreitol; FcRϵ, Fc receptor for IgE; FCS, fetal calf serum; GARG, goat anti-rabbit IgG; IBF, immunoglobulin-binding factor; Ig, immunoglobulin; Igh, immunoglobulin heavy chain locus; i.p., intraperitoneally; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; OVA, ovalbumin; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PDP-protein, 3-(2-pyridyldithio) propionyl protein derivative; RAG, ragweed; RAMA, rabbit anti-mouse IgA antiserum; RAME, rabbit anti-mouse IgE antiserum; RAMG, rabbit anti-mouse IgG antiserum; RIA, radioimmunoassay; r.t., room temperature; SFA, suppressive factor of allergy; SPDP, *N*-succinimidyl 3-(2-pyridyldithio) propionate; SRBC, sheep erythrocytes; T cell, thymus-derived cell; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

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The experimental manipulation of a given type of immune response, either by eliminating the relevant B or T cell components early in their development or by provoking the development of negative control mechanisms early in ontogeny, has always yielded valuable information about the immune system (24, 25). Recently, we have described a phenomenon in which neonatal treatment of mice with soluble or cell-bound monoclonal IgE molecules results in the development of profound IgE class-restricted immunodeficiency (26). Such findings indicate that some cellular component(s) associated with the IgE antibody system, possibly capable of reacting specifically with ϵ determinants of IgE heavy chains (e.g., FcRϵ^+ cells), are perturbed when confronted by IgE at an early stage of development; this early perturbation manifests itself in adulthood as persistent unresponsiveness in the IgE antibody class.

In the present report, we have further explored the IgE class-restricted tolerance model in order to develop some understanding of the mechanisms underlying defective IgE antibody synthesis in this system. Herein, we demonstrate that (*a*) the IgE class-restricted tolerance-inducing signal is, indeed, conveyed by the Fc domain(s) of IgE molecules; (*b*) the ability to develop isotype-restricted tolerance appears to be unique to the IgE antibody system, since neonatal treatment with other immunoglobulin (Ig) isotypes, which as IgA or IgG, does not result in corresponding isotype-restricted immunodeficiency; (*c*) spleen cells from IgE isotype-tolerant mice fail to express FcRϵ following stimulation in vitro with monoclonal IgE, a defect that distinguishes such tolerant mice from their normal control counterparts; and (*d*) neonatal treatment with soluble IgE generates IgE class-specific suppressor T cells whose activity can be readily demonstrated in adoptive transfer experiments, whereas tolerance induction with cell-bound IgE appears to involve a different mechanism, the nature of which is unresolved.

Materials and Methods

Reagents

Proteins and Chemicals. The following materials employed were from commercial sources: 2,4,6-trinitrobenzene sulfonic acid (TNBS; ICN Pharmaceuticals, Cleveland, OH); keyhole limpet hemocyanin (KLH; Pacific Bio-Marine Co., Venice, CA); bovine serum albumin (BSA), ovalbumin (OVA), and dithiothreitol (DTT) (Sigma Chemical Co., St. Louis, MO); CNBr-activated Sepharose-4B and *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (Pharmacia Fine Chemicals, Uppsala, Sweden). DNP_{28}-BSA was prepared as previously described (27).

Monoclonal and Conventional Antibody Reagents. Monoclonal anti-DNP antibodies of the IgE, IgG_1, and IgG_{2b} classes were obtained and purified from hybridomas constructed and characterized previously (27); that of the IgA class was from a myeloma line, MOPC 315. The ragweed (RAG)-specific IgE monoclonal antibody was obtained and purified as described recently (28). Rabbit anti-mouse IgA (RAMA) was purchased from Litton Bionetics, Kensington, MD. Other conventional antibodies were prepared in our laboratory and purified by affinity chromatography on specific immunoadsorbent columns. These included murine anti-KLH specific antibodies, rabbit anti-mouse IgG (RAMG), rabbit anti-mouse IgE (RAME), goat anti-rabbit immunoglobulin (GARG), rabbit anti-KLH, and rabbit anti-BSA.

Animals

(BALB/c × SJL)F_1 mice were obtained from the breeding colonies in the animal quarters of the Medical Biology Institute. Parental strains of female BALB/c and male

SJL mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Adult male Lewis rats were obtained from Holtzman Co., Madison, WI.

Covalent Conjugation of Monoclonal Antibodies to Splenocytes with the Heterobifunctional Reagent, SPDP

The procedure was described in detail previously (26). Briefly, 50 μl of freshly prepared solution (20 mM) of SPDP was added to 1 ml solution of DNP-specific monoclonal IgE, IgG_1, IgG_{2b}, or IgA (1 mg/ml) in phosphate-buffered saline, pH 7.5 (PBS). The mixture was stirred gently for 30 min at room temperature (r.t.), then the excess SPDP reagent was removed by dialysis, and the resulting 3-(2-pyridyldithio) propionyl protein derivative (PDP-protein) was recovered. To generate active sulfhydryl groups on the splenocyte surface, 0.5 ml of 0.01 M DTT was added to 10 ml of 2% spleen cell suspension and the mixture was incubated at r.t. for 1 h. The DTT-treated cells were washed thoroughly with PBS, resuspended in 10 ml of PBS, and then reacted with PDP-proteins (1 mg/ml, 10 ml) at r.t. for 1 h. Protein-coupled splenocytes were washed twice in PBS and resuspended at appropriate concentrations in saline for injection. The quantity of IgE molecules coupled to spleen cells was determined by radiolabeling tracer studies using ^{125}I-labeled PDP-IgE (26).

Adoptive Transfer System

The adoptive transfer system employed for IgE antibody responses was as described by Hamaoka et al. (29). Briefly, donor mice for KLH-immune spleen cells were prepared by immunizations with 1 μg KLH in 2 mg alum injected intraperitoneally (i.p.) at 2–3 wk intervals. In an adoptive transfer, 15×10^6 KLH-immune spleen cells together with 30×10^6 spleen cells, B cells, or T cells, prepared from mice treated neonatally with IgE or control materials, were injected intravenously to syngeneic recipients, which had been irradiated (650 rad) in a ^{137}cesium irradiator on the same day. Immediately after cell transfer, recipients were boosted with 1 μg KLH in 2 mg alum. Mice were bled 7 and 14 d later, and their sera assayed for KLH-specific IgG and IgE antibodies.

Assessment of Antibody Responses

Measurement of Anti-KLH IgA Antibodies. KLH-specific IgE antibody levels in pools of sera from each group were determined by passive cutaneous anaphylaxis (PCA) reactions, using male Holtzman rats as described in detail (29, 30).

Measurement of Anti-KLH IgG and IgA Antibodies. KLH-specific IgG and IgA antibody levels in individual sera, were determined by a solid-phase radioimmunoassay (RIA) system in which serum samples were allowed to react with KLH on solid-phase, and then detected with either RAMG or RAMA, followed by ^{125}I-labeled GARG as previously described in detail (26, 27). For measuring IgA, DNP-specific MOPC 315 IgA myeloma proteins were used as standards. Geometric means and standard errors were obtained from at least four individual mice in each group. Statistical differences were derived by Student's *t* test between treated and control groups.

Preparation of T Cell– and B Cell–enriched Lymphocyte Populations

T cell–enriched lymphocytes were prepared by the nylon wool method (31). B cell–enriched lymphocytes were prepared by treating lymphocytes twice sequentially with anti–Thy-1.2 ascites (C3H anti-AKR) plus C as previously described (32).

In Vitro System for Induction of $FcR\epsilon^+$ Lymphocytes

The procedure was described in detail (14). Briefly, 10^7 splenic lymphocytes from (BALB/c × SJL)F_1 mice, neonatally treated with IgE or control materials, were cultured in vitro in the presence of monoclonal antibodies at varying final concentrations for various lengths of time. Triplicate cultures were pooled, washed twice, and resuspended at 5×10^7 cells/ml. $FcR\epsilon^+$ lymphocytes were detected as cells that form rosettes with IgE-coated sheep erythrocytes (SRBC; reference 33) as described previously (14).

Results

Induction of IgE Class-restricted Tolerance by Neonatal Exposure of (BALB/c × SJL) F_1 Mice to Soluble Monoclonal IgE Antibodies of Two Distinct Antigen Specificities. (BALB/c × SJL)F_1 mice were injected i.p. with various doses of DNP-specific or RAG-specific monoclonal IgE antibodies from 0.25 μg to 250 μg per injection on day 0 and day 6 after birth in 50 μl saline and in 100 μl saline on day 13 and day 37. Control mice were treated with saline. At 3 months of age, these mice were immunized i.p. with 1 μg of KLH in 2 mg of alum and boosted likewise on day 10 and day 24 thereafter; 10 d after the third immunization, sera were collected and analyzed for KLH-specific IgE and IgG antibody responses.

Three points can be made from the results of this experiment, summarized in Fig. 1. First, as shown in *A*, IgE-restricted tolerance was induced with as little as 2.5 μg of soluble IgE per injection; this dose is 100-fold less than the 250 μg of soluble IgE used in our first report (26).

Second, the tolerance-inducing property resides in the constant region of the ε heavy chain. Thus, despite the fact that DNP-specific and RAG-specific monoclonal IgE antibodies have very different antigen-binding specificities, they are equally effective in inducing IgE-restricted tolerance over a 3 log dose range. Third, this tolerance is restricted to the IgE antibody system, since KLH-specific IgG antibody responses were not significantly different between saline-treated or IgE-treated mice (*B*).

IgE Class-restricted Tolerance Can Be Induced by Neonatal Exposure of (BALB/c × SJL)F_1 Mice to Subnanogram Quantities of Syngeneic Cell-bound IgE. Previously, IgE class-restricted tolerance was induced with 25–50 × 10^6 IgE-conjugated cells carrying 5–10 ng of monoclonal IgE per injection (26). Since results in Fig. 1 document that tolerance can be induced with considerably less soluble IgE than heretofore realized, the following experiment was conducted to determine the minimum quantity of cell-bound IgE required for tolerance induction.

(BALB/c × SJL)F_1 mice were injected i.p. with various numbers, ranging from 1 to 50 × 10^6, of DNP-specific IgE-conjugated, or control untreated, syngeneic spleen cells on day 0 and day 10 after birth in 50 μl saline and on day 17 in 100 μl saline. At 2 months of age, they were immunized i.p. with 1 μg of KLH in 2 mg of alum and boosted on day 10 and day 24 thereafter. Sera of these mice were collected on day 34, i.e., 10 d after the third antigen immunization, and analyzed for KLH-specific IgE and IgG antibody responses.

As shown in Fig. 2, all three control groups developed both IgE and IgG antibody responses irrespective of whether they were sham-treated perinatally with saline (group I) or with two different doses of unconjugated syngeneic spleen cells (groups II and III). In contrast, mice treated with IgE-conjugated cells were selectively unresponsive, in the IgE class, even at the lowest cell number employed (1 × 10^6), which corresponds to an absolute amount of 0.2 ng of monoclonal IgE per injection. Thus, by comparison to the results presented in Fig. 1, where at least 2.5 μg of soluble IgE per injection was required for induction of IgE class-restricted tolerance, cell-bound IgE is effective at a dose 10^4 lower than the amount of soluble IgE needed to accomplish the same results.

Isotype-restricted Tolerance by Neonatal Exposure of (BALB/c × SJL)F_1 Mice to

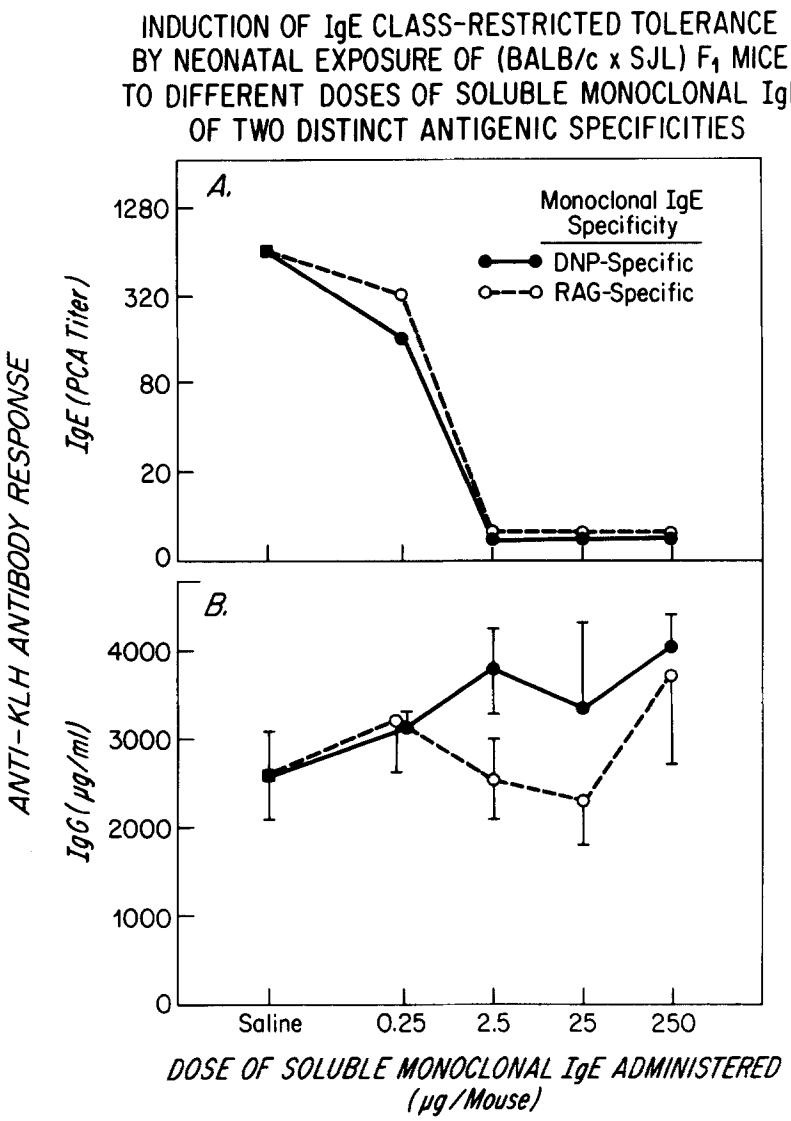

FIGURE 1. Induction of IgE class-restricted tolerance by neonatal exposure of (BALB/c × SJL)F_1 mice to different doses of soluble monoclonal IgE of two distinct antigenic specificities. (BALB/c × SJL)F_1 mice were injected i.p. with various doses of DNP- or RAG-specific monoclonal IgE in 50 μl saline on day 0 and day 6 after birth and in 100 μl saline on day 13 and day 37. At 3 months of age, they were immunized i.p. with 1 μg of KLH in 2 mg of alum and boosted on day 10 and day 24 thereafter. Sera of these mice were collected on day 34 and analyzed for IgE and IgG anti-KLH antibody responses. IgE antibody responses were determined from pooled sera in experimental groups consisting of 5–10 mice each. Individual IgG antibody responses were measured by RIA. Concentrations of IgG anti-KLH antibodies are expressed as geometric means ± SE of the means of individual values from each group; no statistically significant differences exist among the groups.

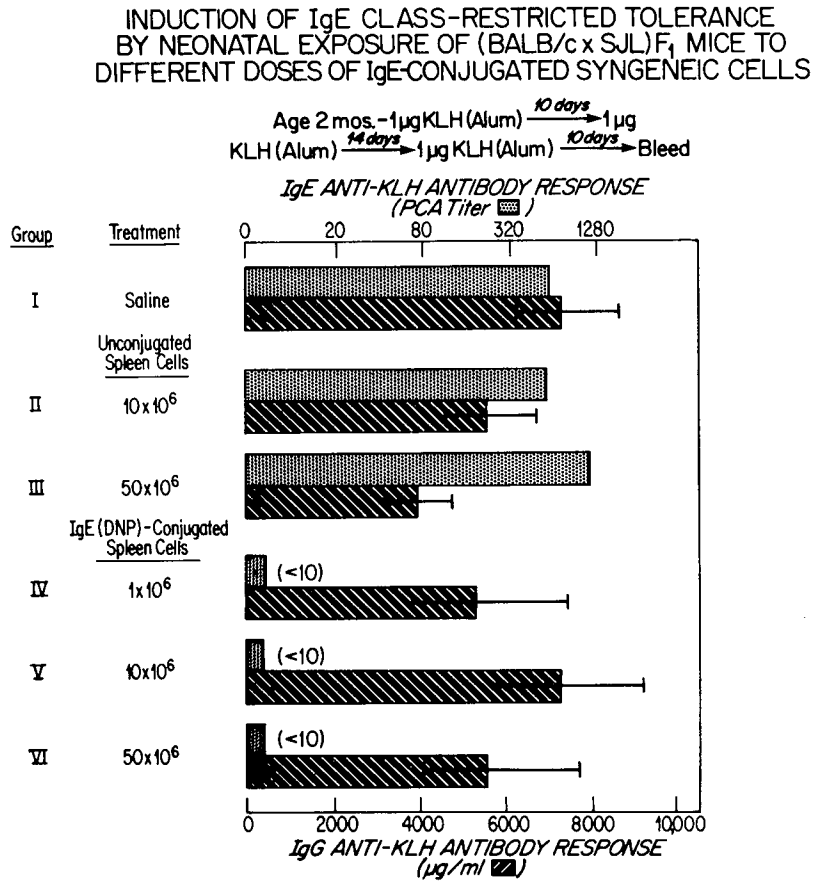

FIGURE 2. Induction of IgE class-restricted tolerance by neonatal exposure of (BALB/c × SJL)F_1 mice to different doses of IgE-conjugated syngeneic cells. (BALB/c × SJL)F_1 mice were injected i.p. with various numbers of DNP-specific IgE-conjugated syngeneic spleen cells, ranging from 1–50 × 10^6 cells, or control materials in 50 µl saline on day 0 and day 10 after birth and in 100 µl saline on day 17. At 2 months of age, they were immunized i.p. with 1 µg of KLH in 2 mg alum and boosted on day 10 and day 24 thereafter. Sera of these mice were collected on day 34 and analyzed for KLH-specific IgE and IgG antibody responses as described in Fig. 1. Each group consisted of at least 6 mice. Numbers in parentheses depict actual PCA titers, where given.

Monoclonal Antibodies Is Only Induced by IgE and Is Unique to the IgE Isotype. The generality of using a monoclonal antibody of a particular class to induce class-restricted unresponsiveness of the corresponding isotype response was tested by administering monoclonal antibodies of different isotypes to neonates. Thus, (BALB/c × SJL)F_1 mice were injected i.p. with either saline (controls) or with monoclonal antibodies of different classes, either in soluble or cell-bound form, on days 0, 12, and 20 after birth. Only the highest doses of soluble and cell-bound IgE found to be effective in the previous experiments were employed, to avoid negative results based on threshold dose effects. At 2 months of age, all mice were immunized i.p. with 1 µg of KLH in 2 mg of alum and boosted on

day 10 and day 24 thereafter. Sera of these mice were collected on day 34 and analyzed for IgE, IgG, and IgA anti-KLH antibody responses.

As shown in Fig. 3, both induction and subsequent expression of this isotype-specific tolerance are highly selective and specific for the IgE antibody system, and this type of isotype-specific tolerance does not operate in classes of antibody responses other than IgE under these conditions. Mice treated neonatally with soluble IgA, IgG_1, IgG_{2b} (groups III–V), or cell-bound IgA, IgG_1 plus IgG_{2b} (groups VII and VIII) are perfectly capable of producing antibodies of the corresponding classes, i.e., antigen-specific IgA (right panel) and IgG (left panel) responses. Thus, mice treated with soluble IgA (group III) produce levels of IgA comparable to mice treated with either saline (group I) or other types of

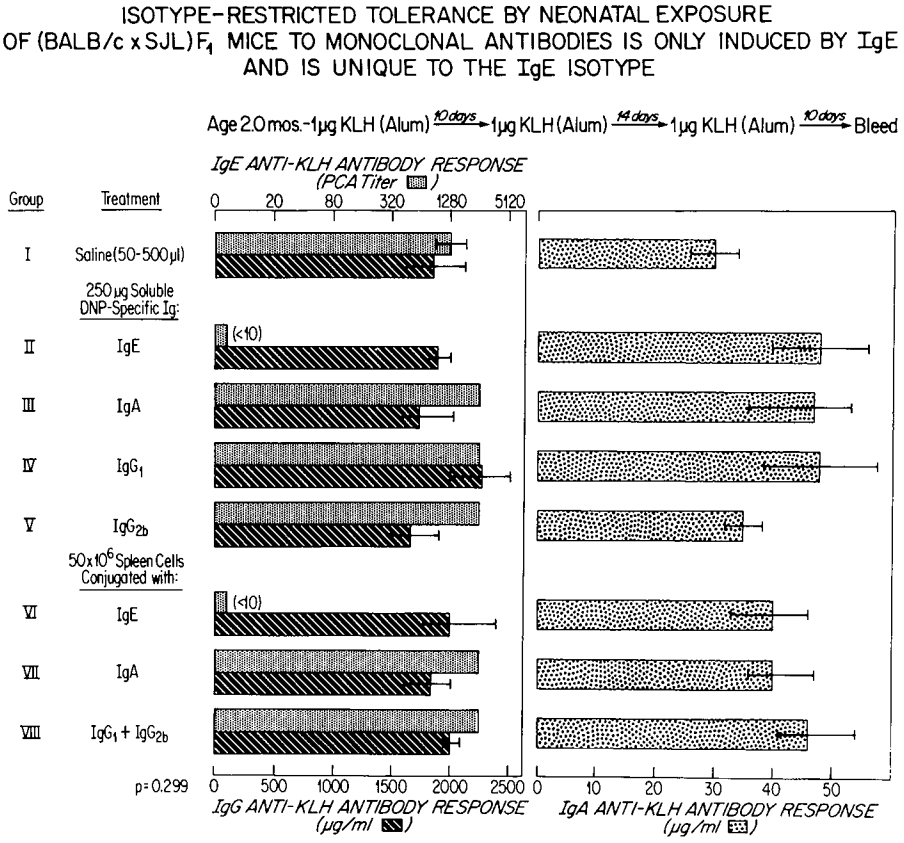

FIGURE 3. Isotype-restricted tolerance by neonatal exposure of (BALB/c × SJL)F_1 mice to monoclonal antibodies is only induced by IgE and is unique to the IgE isotype. (BALB/c × SJL)F_1 mice were injected i.p. with 250 μg of soluble monoclonal antibodies, monoclonal antibodies coupled to syngeneic cells or control materials in 50 μl saline on day 0 and day 12 after birth and in 100 μl saline on day 20. At 2 months of age, they were immunized i.p. with 1 μg of KLH in 2 mg of alum and boosted on day 10 and day 24 thereafter. Sera of these mice were collected on day 34 and analyzed for IgE, IgG and IgA anti-KLH antibody responses. Each group consisted of 5 to 8 mice. IgE antibody responses were determined from pooled sera in experimental groups. IgG and IgA antibody responses were measured by RIA and are expressed as geometric means ± SE of the means of individual values from each group. None of these latter values were significantly different among the groups.

immunoglobulins, i.e., IgE (group II), IgG_1 (group IV), and IgG_{2b} (group V). Likewise, mice treated with IgA coupled to syngeneic cells, and those treated with IgE and IgG_1 plus IgG_{2b} produce similar quantities of IgA (group VII). Furthermore, similar levels of IgG and IgE antibody responses are observed in groups treated with soluble or cell-bound IgA molecules. Similar observations were made for mice treated neonatally with soluble IgG_1 (group IV), IgG_{2b} (group V), or cell-bound IgG_1 plus IgG_{2b} (group VIII), since the total IgG as well as IgA and IgE, antibody responses of these mice are comparable to saline-treated control mice.

The only class of monoclonal antibody that induces isotype-specific tolerance following neonatal administration is monoclonal IgE. Thus, mice treated neonatally with soluble (group II) or cell-bound (group VI) IgE are completely tolerant with respect to their IgE antibody responses, whereas their IgA and IgG antibody response are normal. These data therefore suggest that a constant region domain on IgE molecules uniquely conveys a tolerogenic signal for the IgE antibody system.

In Vitro Induction of $FcR\epsilon^+$ Cells Following Exposure to Monoclonal IgE Is Impaired in Adult Spleen Cells of Mice Treated Perinatally with IgE to Induce IgE Class-restricted Tolerance. In earlier studies from this laboratory (14), we demonstrated that exposure of normal mouse splenic lymphocytes to appropriate concentrations of monoclonal IgE in vitro resulted in the induction of significant numbers of cells expressing IgE-specific Fc receptors, or $FcR\epsilon$. Indeed, these observations, together with the demonstrated ability to modulate such $FcR\epsilon$ expression by concomitant exposure of the same cultures to the IgE class-restricted regulatory molecule, suppressive factor of allergy (SFA), prompted us to initiate the present series of experiments (26). Since the hypothesis leading to the design of such experiments was that $FcR\epsilon^+$ lymphocytes played important regulatory roles in the IgE antibody system, it was thus pertinent to examine whether mice treated perinatally with IgE, and rendered tolerant in the IgE class-restricted fashion, displayed abnormal cellular behavior with regard to in vitro induction of $FcR\epsilon^+$ lymphocytes by exposure to monoclonal IgE.

To make this analysis, the ability of spleen cells from (BALB/c × SJL)F_1 mice treated perinatally with monoclonal IgE to respond to IgE by expression of $FcR\epsilon$ was compared to parallel control groups of F_1 mice. Thus, spleens of mice from groups receiving the same materials three to four times from the experiments described in Figs. 1–3 were used as sources of spleen cells for these studies. All mice, either controls or those treated perinatally with soluble or cell-bound IgE, had been immunized with 1 μg KLH in alum three times at 10–14 day intervals, and were rested for approximately 3 months at the time their spleen cells were removed for the $FcR\epsilon$ induction assay. Single cell suspensions were prepared from a pool of three spleens for each group.

Fig. 4 illustrates the kinetics of responses of such spleen cells to varying doses of IgE in vitro. Splenic lymphocytes (10^7/ml) from different groups of mice were cultured in the presence of different concentrations of monoclonal DNP-specific IgE for 24 h and 48 h, and the frequency of de novo induced $FcR\epsilon^+$ cells was enumerated by their capacity to form rosettes with IgE-coated TNP-SRBC as indicator cells. As shown in Fig. 4*A*, spleen cells from control mice treated

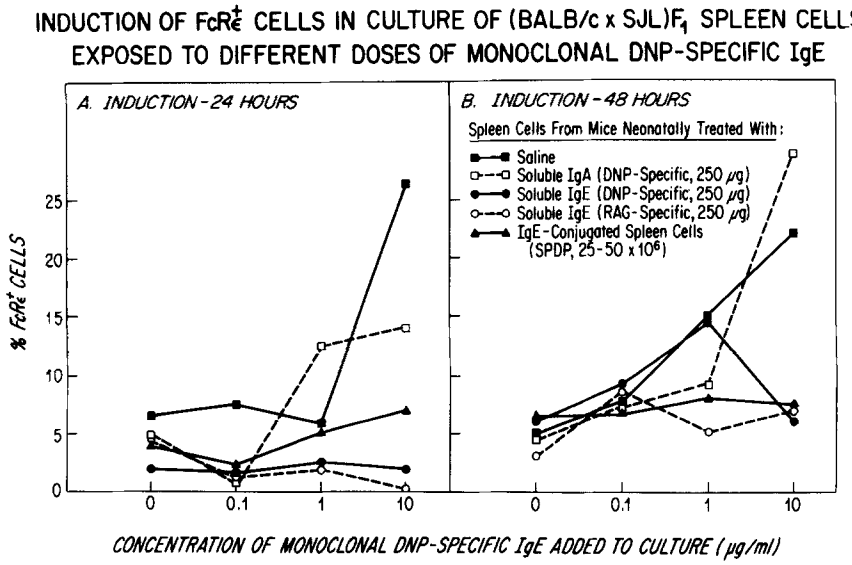

FIGURE 4. Induction of FcRϵ^+ cells in culture of (BALB/c × SJL)F_1 spleen cells exposed to different doses of monoclonal DNP-specific IgE. (BALB/c × SJL)F_1 hybrid mice were injected neonatally with IgE or control material in the experiments summarized in Figs. 1–3. These mice were pooled from groups of mice receiving the same materials 3 to 4 times from the aforementioned three separate experiments. All mice had been immunized with 1 μg KLH in alum 3 times at 10–14-d intervals, and were rested for approximately three months. Mice were about 6 months old at the time of assay. Single cell suspensions were made from spleens of 2–3 mice from each group. 10^7 splenic lymphocytes were cultured in RPMI with 5% FCS in the presence of different concentrations of monoclonal DNP-specific IgE for 24 h and 48 h. The appearance of FcRϵ^+ cells was enumerated by their capacity to form rosette with IgE-coated TNP-SRBC as indicator cells.

neonatally with saline gave rise to 26% FcRϵ^+ cells upon stimulation with 10 μg/ml of IgE in vitro for 24 h. Likewise, spleen cells from mice treated neonatally with soluble IgA manifested clear induction of FcRϵ^+ cells by doses of 1 and 10 μg/ml of IgE. In contrast, spleen cells from mice treated neonatally with 250 μg of soluble IgE of either DNP or RAG specificities, or with 25–50 × 10^6 IgE-conjugated spleen cells were significantly impaired in their ability to express FcRϵ.

As shown in Fig. 4*B*, comparable results were obtained when these spleen cells were incubated for 48 h. When induced with 10 μg/ml of IgE, spleen cells from mice treated neonatally with IgE expressed only 5–7% of FcRϵ^+ cells in contrast to frequencies of 22–28% FcRϵ^+ cells obtained with spleen cells from mice treated with soluble IgA or saline.

Neonatal Exposure of (BALB/c × SJL)F_1 Mice to Soluble IgE Induces Persistent IgE Class-restricted Suppressor T Cells Transferable to Irradiated Adoptive Recipients. The preceding experiment documented that induction of IgE class-restricted tolerance is accompanied by an inability of cells from such mice to develop FcRϵ^+ cells upon in vitro exposure to monoclonal IgE. The absence of such inducible cells could be the direct cause of unresponsiveness in the IgE class, or this could be a secondary manifestation of some other mechanism underlying the unresponsive state. To ascertain whether suppressor cells may be

involved in the described IgE-restricted tolerance, the following experiment was undertaken.

The protocol for this study is summarized on the left portion of Fig. 5, with the corresponding data from each part of the experiment depicted on the right portion of the same figure. As outlined in the top section of the figure, (BALB/c × SJL)F_1 mice were treated on days 0, 7, and 17 after birth with either control or experimental material. Control material consisted of either saline or 50×10^6 unconjugated syngeneic spleen cells, while experimental material consisted of either soluble monoclonal IgE of two different specificities (anti-DNP and anti-RAG, 250 μg per injection) or 50×10^6 IgE-conjugated syngeneic spleen cells. At 2.5 months of age, they were immunized with 1 μg of KLH in 2 mg of alum and boosted on day 10 and day 24 thereafter. Mice were rested for 52 d and boosted with 1 μg KLH in alum. 14 d later, all groups were bled, killed, and their spleens removed to be used as one source of donor cells in the adoptive co-transfer experiment described below. The data summarized in the top portion of Fig. 5 verify that those donor mice treated with either soluble or cell-bound monoclonal IgE displayed long-term IgE class-restricted tolerance that had persisted over a period of six months after receiving only three treatments during the first 3 wk of life.

The adoptive co-transfer experiment, which employed spleen cells from these tolerant mice and their corresponding controls, is summarized in the bottom portion of Fig. 5. The spleens from these donors were pooled by group and prepared as single cell suspensions. Cells from donors treated neonatally with saline or with soluble DNP-specific monoclonal IgE were further fractionated on nylon wool columns to obtain a T cell–enriched fraction or treated with anti-Thy-1.2 plus C to obtain the B cell–enriched fraction. 30×10^6 whole spleen cells, or T cell-enriched or B cell–enriched fractions were then co-transferred intravenously with 15×10^6 whole spleen cells from KLH-hyperimmunized syngeneic donor mice into 650 rad irradiated syngeneic recipients. All recipients were challenged i.p. with 1 μg of KLH in alum and bled 14 d later to assess the resulting IgE anti-KLH antibody responses.

Whole spleen cells from donor mice tolerized by soluble IgE, when injected alone into irradiated recipients, failed to develop IgE anti-KLH antibody responses upon antigen challenge (Table I, groups I–III). As shown in the bottom portion of Fig. 5, co-transfer of spleen cells from donors treated with soluble DNP- or RAG-specific IgE suppressed the adoptive secondary IgE response of the KLH-hyperimmune donor cells, as contrasted to very good responses developed by the same cells when co-transferred with spleen cells from control donors treated neonatally with saline (groups I–III).

Spleen cells from donor mice treated neonatally with unconjugated syngeneic spleen cells developed good IgE anti-KLH antibody responses when they were adoptively transferred alone into irradiated recipients, while donor cells from mice treated with IgE-conjugated cells failed to develop such responses (Table I, groups IV and V). However, as shown in Fig. 5, co-transfer of each of these donor cell types with KLH-hyperimmune spleen cells resulted in equivalent adoptive secondary IgE antibody responses (groups IV and V).

When B cell– and T cell–enriched fractions of spleens from donors treated

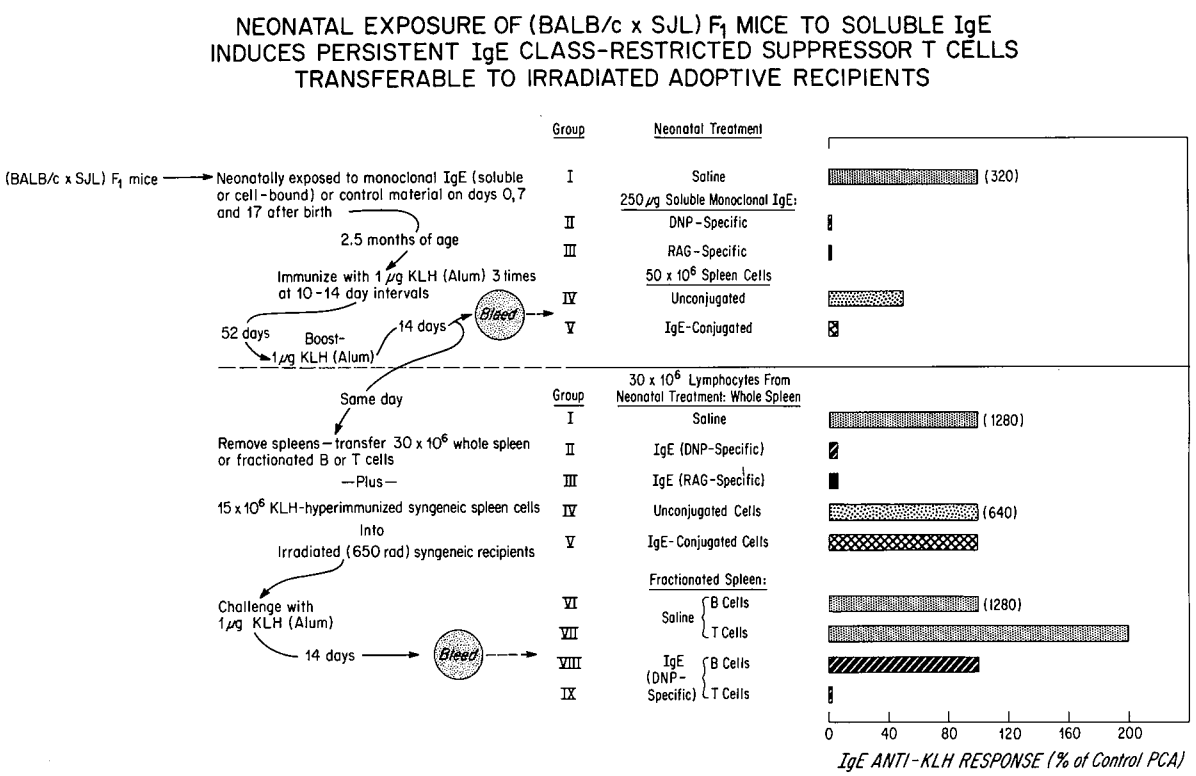

FIGURE 5. Neonatal exposure of (BALB/c × SJL)F_1 mice to soluble IgE induces persistent IgE class-restricted suppressor T cells transferable to irradiated adoptive recipients. (BALB/c × SJL)F_1 mice were treated with monoclonal IgE or control material on days 0, 7, and 17 after birth. At 2.5 months of age, they were immunized with 1 μg of KLH in 2 mg of alum and boosted on day 10 and day 24 thereafter. Mice were rested for 52 d and boosted with 1 μg KLH in alum. The immune status of each group was assessed (top panel). On the same day, 30 × 10^6 whole spleen or fractionated B or T cells were mixed with 15 × 10^6 KLH-hyperimmunized syngeneic spleen cells, and injected into 650 rad irradiated syngeneic recipients and further boosted with 1 μg KLH in alum. 14 d later, mice were bled and the pooled sera were assessed for PCA responses (bottom panel). Data are presented as for IgE responses in Fig. 1, for groups of 4 mice each. Numbers in parentheses depict actual PCA values, where given.

TABLE I

*Spleen Cells from (BALB/c × SJL) F_1 Mice Rendered Tolerant by Neonatal Exposure to Soluble IgE or Cell-bound IgE Are Not Capable of Eliciting IgE Antibody Responses When Transferred into Irradiated Adoptive Recipients**

Group	30×10^6 Whole spleen cells from mice neonatally treated with:	KLH-Specific IgE antibody response (PCA titer)
I	Saline	1,280
II	IgE (DNP-specific)	<10
III	IgE (RAG-specific)	<10
IV	Unconjugated spleen	640
V	IgE-Conjugated spleen cells (DNP-specific)	<10

* (BALB/c × SJL) F_1 mice were treated with monoclonal IgE or control material on days 0, 7, and 17 after birth. At 2.5 months of age, they were immunized with 1 μg of KLH in 2 mg of alum and boosted with 1 μg KLH in alum. 14 d later, 30×10^6 whole spleen cells were injected into 650 rad irradiated syngeneic recipients and boosted with 1 μg KLH in alum. 14 d later, mice were bled and the pooled sera were assessed for PCA responses.

neonatally with either saline or soluble DNP-specific IgE were examined for suppressive activities in this co-transfer system, neither cell type from saline-treated donors exerted any suppressive effects (groups VI and VII), and the T cell–enriched fraction actually caused a twofold enhancement of IgE responses. However, while the B cell–enriched fraction from the soluble IgE-treated donor spleen exerted no detectable effects on the adoptive secondary IgE response (group VIII), the T cell–enriched fraction from these same donor spleens completely abrogated the IgE anti-KLH secondary responses (group IX). It should be emphasized that all of the suppressive activities observed in this experiment (groups II, III, and IX) were selective for responses of the IgE class, since comparable responses among all groups were obtained in the IgG class (data not shown).

Discussion

The experiments presented herein further explored our previous observations on the phenomenon of IgE class-restricted tolerance induced by treatment of neonatal mice with soluble or cell-bound IgE (26). The data obtained from these studies have established the following four points:

Point 1. IgE class-restricted tolerance can be induced in this system by neonatal treatment with monoclonal IgE, irrespective of its antigen specificity, and the resulting effects appear to be polyclonal in nature since IgE responses specific for antigenic determinants unrelated to the tolerance-inducing IgE molecules are affected. Thus, monoclonal IgE antibodies specific for the DNP hapten, on one hand, and for determinants of ragweed antigen, on the other, were comparably effective in inducing this class-restricted tolerance. It is important that the ultimate IgE response tested in such mice as adults was directed to antigenic determinants—in this case, those present on the complex protein, KLH—completely unrelated to the inducing IgE molecules. This formally eliminates any possibility that our previous results (26), which employed only DNP-

specific monoclonal IgE, could have reflected some unsuspected relationships between the DNP-haptenic determinant with dominant determinants on the KLH molecule, and supports the polyclonal nature of this tolerance phenomenon. Moreover, these findings strongly support the likelihood that the molecular subregion(s) involved in inducing IgE class-restricted tolerance in the system resides in the Fcϵ domain of the IgE molecule.

Although not known at present, it will ultimately be important to determine the precise Fcϵ molecular subregion(s) responsible for inducing this tolerance. Liu et al. (34) have cloned the cDNA specifying the ϵ heavy chain fragment of the monoclonal DNP-specific IgE employed in these studies. This cloned cDNA contains the coding regions for part of the Cϵ1 and all of the Cϵ2, Cϵ3, and Cϵ4 domains of the IgE molecule. Comparisons with human IgE reveal that homologies of 36%, 47%, and 51% exist between mouse and human Cϵ2, Cϵ3, and Cϵ4 domains, respectively (34, 35). Two biological observations suggest that inductive signals conveyed by molecular regions in the Fcϵ domains are highly conserved within the mouse and, possibly, between mouse and man: First, the murine DNP-specific IgE antibodies employed in these studies can induce expression of FcRϵ on lymphocytes derived from unrelated strains (C57BL/6 and SJL) of mice (Chen and Katz, unpublished observations). Second, in the aggregated form, this same monoclonal IgE has been shown to induce expression of FcRϵ on human lymphoid cells (36). Whether the conserved molecular subregions of Fcϵ that induce expression of FcRϵ on mouse and human lymphocytes, respectively, are the same as those responsible for inducing neonatal IgE class-restricted tolerance, in the system described herein, has yet to be determined. However, as will be discussed below, some suggestion of this derives from the finding that lymphoid cells from such tolerant mice are unresponsive to IgE-mediated FcRϵ expression.

Point 2. The induction of isotype-restricted tolerance by neonatal treatment with soluble or cell-bound immunoglobulin molecules appears to be a unique feature of the IgE antibody system. Thus, mice treated neonatally with soluble or cell-bound IgA, IgG_1, or IgG_{2b} were perfectly capable of developing antibody responses of the IgA and IgG isotypes, respectively, when immunized as adults. This contrasts with the substantial unresponsiveness, in the IgE class only, manifested by mice treated neonatally with soluble or cell-bound IgE. The reasons for such differences are unknown, but do not necessarily infer something physiologically unique about the IgE system. Rather, since the IgE system is programmed to produce considerably lesser amounts of IgE antibody molecules than is true of other immunoglobulin isotypes, the system may be considerably more sensitive to perturbations conveyed by neonatal treatment in the manner employed in this system.

Point 3. Lymphoid cells obtained from mice treated neonatally with IgE, and subsequently manifesting IgE class-restricted tolerance, do not develop $FcR\epsilon^+$ cells upon in vitro stimulation with IgE, as is characteristic of their untreated counterparts. This failure of such cells to express FcRϵ probably involves both B and T cells, since we have previously shown that in vitro induction with IgE (using normal mouse spleen cells) results in equivalent numbers of $FcR\epsilon^+$ B and T cells (14). Since recent studies in our laboratory have demonstrated that IgE-

mediated in vitro induction of $FcR\epsilon^+$ lymphocytes reflects a cascade of events beginning with B lymphocytes and then subsequently proceeding to T lymphocytes (15–19), this unresponsiveness to $FcR\epsilon$ expression could only be at the B cell level in order to yield the results observed herein; however, it is not possible to determine whether such treatment affects only B cells or both B and T lymphocytes capable of expressing $FcR\epsilon$. In any case, the failure of tolerant spleen cells to express $FcR\epsilon$ following in vitro induction of IgE could reflect one or more of the following events: first, exposure of immature $FcR\epsilon^+$ cells to IgE during the neonatal period could result in irreversible negative modulation of such Fc receptors. Alternatively, such exposure could render such $FcR\epsilon^+$ cells residually capable of expressing $FcR\epsilon$, but only at a low density that would then be insensitive to detection by the rosette assay employed. Second, neonatal exposure to IgE could induce suppressive regulatory cells which, when subsequently exposed to IgE, might prevent the development of $FcR\epsilon^+$ cells in this system. This is reminiscent of earlier observations reported by us demonstrating that the IgE-selective regulatory factor, suppressive factor of allergy (SFA) quite effectively inhibits IgE-mediated $FcR\epsilon$ expression in vitro (14, 16, 19, 37).

Although it is not possible at this time to draw conclusions about the relationship between lack of inducible $FcR\epsilon^+$ cells and the IgE isotype-restricted tolerance phenomenon, we believe that the corollary observations are important and very likely related to one another. Considerable evidence has been recently accumulated indicating that FcR^+ cells participate significantly in regulating isotype-specific antibody responses. Suppressor T cells and their released soluble products, some of which are immunoglobulin-binding factors (IBF), that selectively inhibit synthesis of IgG_1, IgG_{2a}, and IgG_{2b} have been demonstrated by other investigators (38, 39). Similarly, in the IgA system $FcR\alpha^+$ T cells generated in high quantities in IgA myeloma-bearing mice, have been shown to regulate the synthesis of IgA (40). In the IgE system, $FcR\epsilon^+$ cells, and their secreted soluble products, have been shown to positively or negatively regulate various aspects of the IgE antibody system (7–19, 37).

Interestingly, the experimental system described herein reveals an insensitivity, relative to that observed with IgE, to neonatal perturbation by administration of IgA, IgG_1, or IgG_{2b}. As mentioned above, this could reflect the substantial differences in magnitudes of IgE immunoglobulin levels produced in the normal situation as contrasted to quantities of other immunoglobulin isotypes.

Point 4. Mice rendered tolerant by neonatal treatment with soluble IgE possess IgE class-restricted suppressor T cells as a result of the neonatal treatment regimen; no detectable suppressor T cells were observed in mice treated with cell-bound IgE. This may indicate that two different mechanisms underlie the IgE class-restricted tolerance observed in this model. One possible explanation is that IgE-specific helper T cells are somehow affected in mice treated with cell-bound IgE since (*a*) such mice do not respond to antigen challenge in situ, and (*b*) their cells remain unresponsive when adoptively-transferred to irradiated syngeneic recipients (Table I). Although IgE class-specific suppressor cells cannot be demonstrated by co-adoptive cell transfer techniques (Fig. 5), it is possible that cell-bound IgE may be able to elicit suppressor T cells which, together with

suppressive factors that they may produce, could maintain the unresponsive phenotype in situ.

On the other hand, mice treated neonatally with soluble IgE are unresponsive to in situ antigen challenge, but also possess IgE class-restricted suppressor T cells demonstrable in adoptive transfer circumstances. It is possible that neonatal treatment with soluble IgE more effectively generates transferable suppressor T cells as a result of an interaction between fluid-phase IgE and $FcR\epsilon^+$ precursor cells resulting in an IgE-$FcR\epsilon^+$ cell surface complex which, in turn, may induce second-order suppressive regulatory cells. Since $FcR\epsilon^+$ cells cannot be induced in lymphoid populations from such tolerant mice, we favor the interpretation that these second-order suppressor cells, once induced, may be maintained in such animals and actively suppress the development of IgE antibody-forming cells and/or IgE class-specific helper T cells via receptors directed against the $FcR\epsilon$ itself.

This experimental system of IgE isotype-restricted tolerance provides a different avenue for exploring the composition and complex cellular interactions regulating the IgE antibody system. Delineating the precise tolerance-inducing molecular sites on the IgE molecule and the mechanism by which tolerogenic signals are delivered could ultimately help to unravel molecular mechanisms controlling IgE antibody synthesis and, perhaps, synthesis of other immunoglobulin isotypes as well.

Summary

Certain aspects of the phenomenon of IgE class-restricted tolerance induced in mice by neonatal treatment with monoclonal IgE, either in soluble form or coupled to syngeneic spleen cells, were examined. The present studies document that this tolerance results from exposure to IgE molecules, irrespective of their antigen specificity, and the resulting effects are polyclonal in nature since IgE responses directed against antigenic determinants unrelated to the tolerance-inducing IgE molecules are affected. Moreover, such findings indicate that the molecular subregion(s) responsible for inducing IgE class-restricted tolerance resides in the ϵ heavy chain constant region domain(s) of IgE. When soluble IgE is employed, tolerance induction results from neonatal treatment with doses as low as 2.5 μg per injection per mouse; cell-bound IgE is considerably more potent, in terms of total dose required, since tolerance results from treatment with as few as 1×10^6 cells per injection (per mouse), equivalent to an absolute quantity of 0.2 ng of IgE per injection. This long-term class-specific tolerance appears to be a unique feature of the IgE antibody system, since treatment of mice with monoclonal antibodies of the IgA, IgG_1, or IgG_{2b} isotypes, either in soluble or cell-bound form, does not perturb antibody responses of their corresponding isotypes or in the IgE class.

By analyzing the lymphoid cells of IgE-tolerant mice after they reached adulthood, the following observations were made: (*a*) lymphoid cells from such tolerant mice fail to develop $FcR\epsilon^+$ cells upon in vitro stimulation with IgE, as is characteristically observed with lymphoid cells from nontolerant mice; and (*b*) mice rendered tolerant by neonatal treatment with soluble IgE possess IgE class-restricted suppressor T cells, demonstrable in adoptive transfer experiments,

whereas no such suppressor cells are evident in mice in which cell-bound IgE was used for neonatal treatment. The latter observations could mean that two different mechanisms underlie the IgE class-restricted tolerance, or both mechanisms operate coordinately to varying degrees depending upon which regimen is used for tolerance induction, as discussed herein.

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