Breakdown of T cell tolerance to IgG2a\textsuperscript{b} in Igh\textsuperscript{a} mice by \textit{de novo} emerging anti-IgG2a\textsuperscript{b} T cells and not anergy reversion

Laleh Majlessi, Nipa Rujithamkul, Odile Burlen-Defranoux\textsuperscript{1} and Guy Bordenave

Unite\'e d’Immunophysiologie Mole\'culaire and \textsuperscript{1}Unite\'e d’Immunobiologie, Institut Pasteur, 25, rue du Docteur-Roux, 75724 Paris Cedex 15, France

Keywords: clonal elimination, Ig allotype, T cell recruitment, thymus

Abstract

The intrinsic T cell activity of Igh\textsuperscript{a} mice against IgG2a\textsuperscript{b} (IgG2a from the Igh\textsuperscript{b} haplotype) can be subjected to profound specific tolerance. \textit{In utero} followed by post-natal exposure of Igh\textsuperscript{a} mice to soluble IgG2a\textsuperscript{b} results in the loss of the capacity of their T splenocytes to induce specific and chronic IgG2a\textsuperscript{b} allotype suppression in histocompatible Igh\textsuperscript{a/b} recipients. However, this full T cell tolerance has not been definitively acquired as it is spontaneously reversed when investigated 3–6 months after the end of the tolerogen treatment. Even when the IgG2a\textsuperscript{b} tolerogen treatment was prolonged to 3, 6 or 9 months of age, T cell tolerance to IgG2a\textsuperscript{b} vanished and the capacity of Igh\textsuperscript{a} T splenocytes to induce IgG2a\textsuperscript{b} suppression in Igh\textsuperscript{a/b} recipients was systematically restored. The marked but partial thymus involution in 15-month-old Igh\textsuperscript{a} mice suggests the existence of some residual thymic output, capable of repopulating the anti-IgG2a\textsuperscript{b} peripheral T pool subsequent to tolerogen clearance. In the present study, we showed that the mechanisms of this tolerance and its reversion involve, at the end of tolerogen treatment, the physical elimination or the irreversible inactivation of natural anti-IgG2a\textsuperscript{b} T cell clones and their replacement, but neither the establishment of reversible anergy nor the recruitment of T cells which could actively maintain tolerance. The spontaneous breakdown of this T cell unresponsiveness was effectively prevented when \textit{de novo} T cell maturation was inhibited by thymectomy at the end of tolerogen administration. Moreover, tolerance reversion did not occur in peripheral mature Igh\textsuperscript{a} T cells, \textit{parked in vivo}, for up to 20 weeks in histocompatible tolerogen-free nu/nu mice.

Introduction

Despite their shared genetic background, mice of the Igh\textsuperscript{a} haplotype display an intrinsic T cell activity against IgG2a from the Igh\textsuperscript{b} haplotype (IgG2a\textsuperscript{b}) (1), while their Igh\textsuperscript{b} congenic counterparts are tolerant to this self antigen and express substantial amounts of this Ig allotype. Indeed, normal or \textit{a fortiori} specifically stimulated T splenocytes from Igh\textsuperscript{a} mice are able to induce complete and chronic IgG2a\textsuperscript{b} suppression in histocompatible Igh\textsuperscript{a/b} or Igh\textsuperscript{b} congenic recipients (1–3 and reviewed in 4). This asymmetrical behaviour of Igh\textsuperscript{a} and Igh\textsuperscript{b} mice in this respect provides a non-transgenic and non-superantigenic model favourable to the study of T cell tolerance to IgG2a\textsuperscript{b} and of the involved TCR repertoire, which differs between Igh\textsuperscript{a} and Igh\textsuperscript{b} congenic mice.

In this Ig-allotype suppression system, cellular collaboration between the donor’s anti-IgG2a\textsuperscript{b} CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells is required during the suppression-induction phase (5,6) but only CD8\textsuperscript{+} T cells, always of donor origin (7), are necessary for suppression maintenance (3). C.2a\textsuperscript{b} peptide presentation to suppression-inducer/effecter T cells (8) necessitates expression of MHC class I (but not class II) molecules by the recipient’s IgG2a\textsuperscript{b+} target B cells (9).

We have previously demonstrated that stringent perinatal B cell deprivation in Igh\textsuperscript{a/b} or Igh\textsuperscript{b} mice resulted in the emergence of anti-IgG2a\textsuperscript{b} T cells (10) which spontaneously and progressively induced an autoimmune and chronic IgG2a\textsuperscript{b} suppression presenting all the properties of the T cell-induced suppression that occurred by transfer of T splenocytes from Igh\textsuperscript{a} mice into adequate Igh\textsuperscript{b} or Igh\textsuperscript{a/b} hosts. Conversely, perinatal exposure of Igh\textsuperscript{a} mice to the soluble form of IgG2a\textsuperscript{b} allotype (but not to irrelevant Ig) induced full and specific tolerance to this allotype (10). In Igh\textsuperscript{a} mice, T cell tolerance to IgG2a\textsuperscript{b} is characterized \textit{in vivo} by the
incapacity of the IgG2a b-primed T splenocytes to induce IgG2a b suppression in IgG2a b recipients. We recently examined in detail the maintenance of this tolerance and showed that this total unresponsiveness was, however, not definitively acquired but progressively reversed after IgG2a b clearance (11). Even when IgG2a b mice were continuously exposed to the tolerogen from the perinatal period to 3 or 6 months of age, the anti-IgG2a b T cell activity was restored 3 months after the end of tolerogen treatment. The course of tolerance reversion was only slightly delayed when the tolerogen treatment was prolonged in these mice until 9 months of age, when the thymus showed marked but partial involu
tion.

It had not been previously established whether this tolerance induction was due to mechanisms of clonal deletion (12–16), functional unresponsiveness (anergy) (17–19) or active cell-mediated inhibition of specific clones (20–23). The systematic reversion of the T cell tolerance induced by soluble IgG2a b in IgG2a mice enabled these possibilities to be examined in the present investigation. To determine responsibility, we followed two strategies: (i) prevention of de novo emergence of anti-IgG2a b T cells, in IgG2a mice, by thymectomizing them at the end of tolerogen treatment, and (ii) long-term in vivo deposition of T splenocytes from tolerant IgG2a mice in an athymic tolerogen-free and histocompatible environment “in vivo parking" (24). The tolerance or responsiveness to IgG2a b was then physiologically assessed, after amplification of the possible anti-IgG2a b T cell activity (sensitization), by adoptive transfer of the resultant T splenocytes (Tsens) into histocompatible IgG2a b F1, born to IgG2a congenic parents, in which the serum IgG2a b production was regularly measured.

Methods

Mice

BALB/c (Igh a), wild-type or nu/nu mice were obtained from Iffa Credo (L’Arbresle, France). The CB20 (Igh b) mouse, raised in the Pasteur Institute’s facilities, are IgG2a b congenic to the BALB/c strain and have the IgG2a b region of C57BL/6 mice. We bred BALB/c × CB20 (Igh a/b) F1 by crossing BALB/c (Igh a) females with CB20 (Igh b) males.

Mouse sensitization and suppression induction

To amplify their possible anti-IgG2a b T cell activity, BALB/c (Igh a) mice (wild-type or nu/nu) in each experimental group received, i.e., at a 15 day interval, 5 × 10^7 living B splenocytes from CB20 (Igh b) mice, prepared by in vitro cytotoxic anti-Thy-1.2 (30-H-12) mAb (25) treatment in the presence of guinea pig serum as the source of C. Seven days after the second injection, splenocytes from wild-type sensitized BALB/c mice were passed through nylon-wool columns (26) to obtain a T lymphocyte-enriched population (Tsens). Splenocytes from normal or sensitized nu/nu mice were used without further treatment. The IgG2a b suppression-induction was assayed in BALB/c × CB20 (Igh b) newborns by injecting i.p. 1 × 10^7 living Tsens/50 µl or 5 × 10^7 living splenocytes/50 µl. For serum IgG2a b expression studies, IgG2a b F1 untreated controls and cell recipients were bled regularly from the retro-orbital plexus between 7 and 34 weeks of age. Serum allotype detection and quantification have been fully described elsewhere (11). Briefly, the presence of serum IgG2a b was first determined by immunoprecipitation in 1% agar-gel medium using a BALB/c anti-IgG2a b serum with a detection limit of 5 µg/ml. The IgG2a b concentration was then measured, using anti-IgG2a b 5.7.2 mAb (27), in an ELISA with a detection limit of 0.05 µg/ml. Using this ELISA, only mice with undetectable serum IgG2a b were considered to be subjected to suppression.

Preparation of the IgG2a b tolerogen and tolerance-induction procedure

The mouse myeloma CBPC101 (IgG2a b) cell line was a gift from Dr M. Potter (National Cancer Institute Contract N-01-CB-71 085). The myeloma IgG2a b was semi-purified from ascitic fluid by 18% Na 2SO4 precipitation followed by gel filtration through Sepharose 6B. This semi-purified IgG2a b preparation, used for tolerance induction, was spun at 10,000 r.p.m. and filtered through a 0.2 µm membrane, and therefore did not contain microaggregates.

The perinatal tolerogenesis consisted of in utero followed by post-natal exposure of IgG2a b mice to soluble IgG2a b. This protocol was inspired by that applied to obtain B cell deprivation by perinatal administration of anti-IgM antibodies (28). For in utero tolerogen treatment, during the last week of gestation, timed-pregnant BALB/c females received, i.p., 5 mg of IgG2a b/250 µl/day for Ig transmission to their offspring through the placental barrier. For tolerogen transmission by nursing during the lactation period (3 weeks), mothers continued to be injected, i.p., with 1 mg of IgG2a b/250 µl/day. Their IgG2a progeny received, i.p., 1 mg of IgG2a b/50 µl/day for 3 weeks and then, after weaning, 7 mg of IgG2a b/300 µl/week for a further 3 weeks.

Thymectomy

Six-week-old BALB/c mice, untreated controls or perinatally IgG2a b treated, were thymectomed by vacuum aspiration after sternum incision under general anaesthesia obtained by an i.p. injection of 250–300 µl of Avertin (2.5% tert-amyl alcohol in distilled water, 12.5 µg/ml 2,2,2-tribromo ethanol; Aldrich, Steinheim, Germany). Total thymus removal was confirmed anatomically by dissection when the thymectomized mice were sacrificed at the age of 6 months for transfer of their T splenocytes into IgG2a b F1. Mice were excluded when such confirmation was dubious.

T cell transfers into BALB/c nu/nu mice

Living T cell-enriched splenocytes (5 × 10^7) from untreated controls or perinatally IgG2a b-treated BALB/c mice were injected, i.e., into 7-week-old BALB/c nu/nu mice. Reconstitution of their T cell compartment was followed by FACS analysis of their peripheral blood lymphocytes or splenocytes.

mAb origin and FACS analysis

FITC-conjugated anti-CD3ε mAb (29) was kindly provided by Dr P. Trufa-Bachi (Pasteur Institute, Paris). Phycoerythrin-conjugated anti-CD4 (YTS 191.1) and anti-CD8 (YTS.169.4) mAb were purchased from Caltag (South San Francisco, CA). Viable labeled cells were analyzed by acquiring 10,000 events after setting gates on forward/side scatter and propidium
iodide$^-$ cells in a FACScan system (Becton Dickinson, Mountain View, CA) by using Cell Quest software.

Statistical analysis

Statistical significance of percentage differences was determined by $\chi^2$ test. For mean comparison between, each time, two different groups, the significant equality of their variances was first verified by fluctuation $F$-test ($P < 0.05$). The involved means were then compared statistically by Student’s $t$-test (30).

Results

Spontaneous breakdown of T cell tolerance to IgG2a$^b$ is blocked by preventing de novo T cell maturation after the end of the tolerogen treatment

To induce specific tolerance to the IgG2a$^b$ allotype, BALB/c IgH$^a$ mice were perinatally treated with soluble IgG2a$^b$, from the last week of the fetal period to the age of 6 weeks. Such tolerogen-treated individuals will be referred to as IgG2a$^b$ pn mice. We first checked, in a sample population of BALB/c IgG2a$^b$ pn mice, that, at the age of 3 months, the tolerance to IgG2a$^b$ was effectively established. For this purpose, age-matched IgG2a$^b$-untreated and IgG2a$^b$ pn BALB/c mice were sensitized with B splenocytes from IgH$^b$ congenic CB20 to amplify their possible anti-IgG2a$^b$ T cell activity. The resultant Tsens were adoptively transferred into BALB/c x CB20 (Igh$^{ab}$) newborns in which suppression induction and maintenance were subsequently followed during adulthood. As shown in Table 1, while Tsens from tolerogen-untreated individuals induced chronic suppression in 100% of their IgH$^{ab}$ recipients (n = 30), Tsens from IgG2a$^b$ pn mice strongly and significantly [assessed using the $\chi^2$ test ($P < 0.05$)] lost their suppression-induction capacity, as they induced suppression only in a maximum of 4.2% of their IgH$^{ab}$ recipients (n = 24). This result shows that, in this experimental group, the T cell tolerance to IgG2a$^b$ was effectively established at the age of 3 months.

We then investigated the persistence of this perinatally induced tolerance when the emergence of new T cells was blocked, by thymectomizing the 6-week-old mice, 24 h after the last injection of the soluble tolerogen. The anti-IgG2a$^b$ T cell suppression activity in these IgG2a$^b$ pn BALB/c mice either thymectomized since the age of 6 weeks (referred to as IgG2a$^b$ pn/Tx) or non-thymectomized was then studied when they reached 6 months of age. We also included two groups of tolerogen-untreated age-matched BALB/c mice: in the first, mice were thymectomized at the age of 6 weeks; in the second, they were not thymectomized. All these mice were sensitized and the possible suppression-induction capacity of their Tsens was studied by transfer into IgH$^{ab}$ F1, CD3$^+$ CD4$^+$ and CD3$^+$ CD8$^+$ T subset percentages, determined by FACS analysis of splenocytes or Tsens from thymectomized or non-thymectomized IgG2a$^b$-treated or IgG2a$^b$-untreated IgH$^a$ donors, were comparable (data not shown). As illustrated in Fig. 1, Tsens from IgG2a$^b$ untreated euthymic or Tx IgH$^a$ mice induced suppression in 100% of their recipients (n = 29 and n = 21 respectively). The comparable capacity of Tsens from IgG2a$^b$ untreated euthymic and Tx IgH$^a$ mice to induce suppression and their equivalent T subset percentages are consistent with the fact that post-thymic behaviour of T lymphocytes, exported to the peripheral lymphoid organs, is thereafter independent of thymic output (31). Tsens from euthymic IgG2a$^b$ pn mice exhibited a highly significant [assessed by $\chi^2$ test ($P < 0.05$)] loss of tolerance to IgG2a$^b$ and induced suppression in up to 80% of their IgH$^{ab}$ recipients (n = 25) while Tsens from IgG2a$^b$ pn/Tx remained firmly tolerant to IgG2a$^b$ and were unable to induce suppression in IgH$^{ab}$ F1 (n = 12). Among untreated controls, no individual was subjected to IgG2a$^b$ suppression (n = 14).

The mean IgG2a$^b$ concentration in IgH$^{ab}$ recipients of Tsens from 6-month-old euthymic IgG2a$^b$ pn mice (5.2 ± 11.0 µg/ml) was markedly lower than that of untreated IgH$^{ab}$ controls (103.9 ± 30.0 µg/ml) or that of recipients of Tsens from age-matched IgG2a$^b$ pn/Tx mice (71.2 ± 33.1 µg/ml). As determined by Student’s $t$-test ($P < 0.05$), this latter mean was only slightly but significantly lower than that of untreated controls. In accordance with the suppression-induction incapacity or capacity of respectively IgG2a$^b$ pn/Tx or IgG2a$^b$ untreated Tx mice, the sensitizing IgG2a$^b$ B cells perfectly engrained and produced this allotype in the former but not in the latter (data not shown).

Consequently, these data demonstrated that the tolerance to IgG2a$^b$ became chronic in IgG2a$^b$ pn IgH$^a$ mice when de novo T cell maturation and differentiation were prevented by thymectomy after the end of the tolerogen treatment.

Unresponsiveness of peripheral mature T cells is not reversed by long-term deposition in an athymic tolerogen-free environment

In parallel to thymectomy experiments, we studied the persistence of the induced tolerance to IgG2a$^b$ in a T cell population
Breakdown of T cell tolerance to IgG2ab is prevented by inhibition of de novo T cell emergence after the end of the tolerogen treatment. Serum IgG2ab concentrations in 25-week-old Igha/b mice untreated or recipients of Tsens from Igha mice: tolerogen-untreated non-thymectomized or thymectomized or perinatally tolerogen-treated non-thymectomized or thymectomized. The state of IgG2ab suppression or production remained stable until the age of 34 weeks (last bleeding studied). n represents the number of untreated controls or Igha/b recipients. Vertical bars represent the mean IgG2ab concentrations.

Table 2. T subset reconstitution, in tolerogen-free syngenic nu/nu BALB/c mice, by parked T splenocytes from normal or IgG2ab pn Igha mice

<table>
<thead>
<tr>
<th>BALB/c Igha mice</th>
<th>n</th>
<th>Cells transferred</th>
<th>PBL</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD3⁺CD4⁺ (%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>3</td>
<td>-</td>
<td>54.9 ± 0.6</td>
<td>17.0 ± 2.6</td>
</tr>
<tr>
<td>nu/nu</td>
<td>6</td>
<td></td>
<td>0.5 ± 0.3</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>nu/nu</td>
<td>3</td>
<td>T splenocytes from normal Igha mice</td>
<td>19.9 ± 4.0</td>
<td>9.0 ± 3.4</td>
</tr>
<tr>
<td>nu/nu</td>
<td>5</td>
<td>T splenocytes from IgG2ab pn Igha mice</td>
<td>21.9 ± 3.1</td>
<td>11.4 ± 2.9</td>
</tr>
</tbody>
</table>

Individual FACS analyses were performed on PBL (16 weeks) and on splenocytes (20 weeks) after T cell transfers into 7-week-old nu/nu BALB/c mice. n represents the number of mice studied. PBL, peripheral blood mononuclear cells.

Transferred into tolerogen-free, T cell-deficient mice (in vivo parking strategy), nu/nu Igha BALB/c mice provide a syngenic and IgG2ab-free environment for long-term in vivo deposition of normal or tolerant Igha T cells. At the end of tolerogen treatment, i.e. at the age of 6 weeks, 5×10⁷ T cell-enriched splenocytes from normal or IgG2ab pn Igha mice were transferred into 7-week-old nu/nu BALB/c depositary mice. The repopulation of the T compartments of these nu/nu mice was followed by FACS studies of their peripheral blood lymphocytes, 16 weeks later, and of their splenocytes, 20 weeks later. As shown in Table 2, although these T subset repopulations were partial and the T cell percentages did not attain those of untreated wild-type BALB/c mice, the CD3⁺CD4⁺ and CD3⁺CD8⁺ compartment reconstitutions, with T splenocytes from normal or IgG2ab pn Igha mice, were significantly similar to one another (Student’s t-test (P < 0.05)) and each was markedly higher than that of unreconstituted nu/nu controls.

Twenty weeks after the T cell transfers, the possible anti-IgG2ab T cell activity of in vivo parked T cells was investigated. Untreated or T cell-reconstituted nu/nu mice were sensitized and their suppression-induction capacity was evaluated by transfer of their splenocytes into Igha/b newborns. To apply a transplantation protocol, as homogeneous as possible to our usual regimen, 5×10⁷ total splenocytes from non-reconsti-
Mechanism of T cell tolerance to IgG2a

Fig. 2. The T cell tolerance to IgG2a is irreversible in peripheral mature T cells subjected to perinatal tolerogenesis by soluble IgG2a and parked, for 20 weeks, in syngenic tolerogen-free nu/nu BALB/c mice. Serum IgG2a concentrations in 25-week-old Igha/b mice recipients of sensitized splenocytes from nu/nu BALB/c mice, non-reconstituted or reconstituted with T splenocytes from normal or perinatally tolerogen-treated (IgG2ab pn) Igha BALB/c mice. The state of IgG2a suppression or production remained stable until the age of 34 weeks (last bleeding studied). n represents the number of these Igha/b recipients. Vertical bars represent the mean IgG2a concentrations.

Treated or T cell reconstituted nu/nu mice were transferred into Igha/b newborns, instead of $1 \times 10^7$ T splenocytes. As shown in Fig. 2, splenocytes from sensitized but non-reconstituted nu/nu mice were totally devoid of endogenous anti-IgG2a T cell activity and did not induce suppression in their Igha/b recipients ($n = 24$). This observation is in very good agreement with our earlier finding that this allotype suppression was indeed T cell induced. Upon sensitization, normal Igha T cells, parked for 20 weeks in nu/nu mice, were able to induce suppression in 100% of their Igha/b recipients ($n = 9$), while T cells from tolerant IgG2ab pn Igha mice, parked under the same conditions, remained highly significantly [assessed using the $\chi^2$ test ($P < 0.05$)] tolerant to IgG2a and induced suppression in only 9.1% of their Igha/b recipients ($n = 11$).

The mean IgG2a concentration in Igha/b recipients of in vivo parked normal T cells was $0.00 \pm 0.00$ µg/ml and of course drastically lower than that in recipients of in vivo parked tolerant T cells (53.4 ± 35.0 µg/ml). As analyzed using Student's $t$-test ($P < 0.05$), the mean IgG2a concentration in these latter was slightly but significantly lower than that in Igha/b recipients of splenocytes from non-reconstituted nu/nu mice (92.2 ± 30.4 µg/ml). These events are reminiscent of those of recipients of Tsens from 6-month-old Igha IgG2a pn/Tx mice (Fig. 1). The sensitizing IgG2a B cells engrafted and produced IgG2a in nu/nu mice non-reconstituted or reconstituted with T cells from IgG2a pn BALB/c Igha mice; which is in agreement with the suppression-induction incapacity of these mice. Such IgG2a production was not detected after the sensitization of nu/nu mice reconstituted with normal BALB/c T cells (data not shown).

Consequently, the tolerance to IgG2a induced by the soluble tolerogen does not imply reversible functional paralysis because the unresponsiveness persisted in mature T splenocytes from IgG2ab pn Igha mice throughout long-term (20 weeks) in vivo deposition in an athymic tolerogen-free environment.

Discussion

Over the last few years, experimental animal models of specific T cell-tolerance induction and antigen-specific immunotherapy have essentially used TCR transgenic mice (14,32–34) or superantigenic T cell responses (12,13,35). In this present study, the in vivo T cell-induced IgG2a suppression system (reviewed in 4) was exploited, as a physiological model, to investigate the mechanisms of the full and specific T cell tolerance to IgG2a, and its systematic reversion in mice of the Igha haplotype, perinatally exposed to soluble IgG2a. This model has been developed based on the existence, in untreated normal Igha mice, of an intrinsic T cell activity against the expression of IgG2a from the Igha haplotype. This activity is particularly evident when normal or specifically IgG2a-primed T splenocytes from Igha mice are adoptively transferred into Igha mouse or Igha newborns.

Specific tolerance to IgG2a can be induced perinatally in Igha mice by exposing them to soluble IgG2a from the last
Mechanism of T cell tolerance to IgG2aβ

week of their fetal period to 6 weeks of age (10). The unresponsiveness of these Igha mice to IgG2aβ, established until 3 months of age, results in a drastic and specific loss of their suppression-induction capacity, when their Tsens are transferred into histocompatible Ighbβ recipients. Nevertheless, this T cell tolerance is progressively reversed with tolerogen clearance, i.e. within 3 months after the end of IgG2aβ administration. Furthermore, the acquired tolerance was not definitive, even when the exposure to tolerogen was prolonged to 9 months of age (11). Here, we shed some light on the mechanisms of induction and reversion of this tolerance. Perinatal exposure of Igha BALB/c mice to soluble IgG2aβ followed by thymectomy, after the end of tolerogen treatment, resulted in definitive acquisition of tolerance. Indeed, the systematic re-establishment of anti-IgG2aβ T cell activity was effectively inhibited by preventing de novo T cell differentiation and maturation. It is noteworthy that, by thymectomy 1 day after the last tolerogen injection at 6 weeks of age, we deliberately chose to investigate the reversibility of tolerance in the T cells exposed to the high levels of circulating tolerogen. It will be interesting to study, with this strategy, the behaviour of T cells, generated throughout the period of progressive decrease of tolerogen concentrations, e.g. at the age of 3 months. Nevertheless, in the latter case the distinction between individuals always subjected to tolerance to those having already reversed it will oblige us to carry out individual (rather than pool) Tsens transfers. Moreover, the long-term (20 weeks) in vivo parking of T splenocytes from tolerant Igha mice in a tolerogen-free athymic nu/nu environment demonstrated that the tolerance established in the mature peripheral T cell compartment was irreversible; indeed, it can be considered that these in vivo parked T splenocytes remained unable, upon sensitization, to induce suppression in Ighhβ recipients. These results indicate that the specific T cell tolerance to IgG2aβ, perinatally induced in Ighhα mice by soluble IgG2aβ, involves central or peripheral physical elimination or irreversible inactivation of anti-IgG2aβ T cells and not reversible anergy mechanisms. The reversion of tolerance is directly due to the emergence of new anti-IgG2aβ T cells, migrating to peripheral lymphoid organs, as long as the thymus remains functional. We previously observed that the thymus involution, assessed by the cellularity of this organ, at least in the BALB/c mice we studied, was not complete even at 15 months of age and that the anti-IgG2aβ T cell activity could still be recovered in Ighhα mice exposed to IgG2aβ from the perinatal period to 9 months of age (last time point studied) (11). Despite severe thymus size reduction and slowing down of T cell export with ageing, the thymic output of 24-month-old mice has been estimated to be reduced to 0.7% (but not 0.0%) of that of newborns (36). On the other hand, the expression of the basic anti-IgG2aβ T cell activity in normal Ighhα mice could suggest the existence of a particularly high incidence of VαJα/VβDJβ TCR gene rearrangement for this specificity. The resultant high frequency of anti-IgG2aβ T cell resurgence would then still allow the breakdown of tolerance to IgG2aβ in aged Ighhα mice, despite the reduction of their thymic output, as soon as the tolerogen disappeared. Surely this resurgence also participates in the normal turnover of these T cells.

It is unlikely that, for Ighhα anti-IgG2aβ T splenocytes, a type of irreversible anergy would persist for a long time in the absence of tolerogen, after thymectomy or during in vivo parking, because T cell tolerance by anergy has been shown, in several experimental systems, to undergo in vivo reversion in the absence of tolerogen. (i) The in vitro T cell proliferation in response to anti-TCR Vβ mAb, intensely inhibited by in vivo superantigen-induced anergy, was totally restored after 3 weeks of parking in superantigen-free mice (24). (ii) Anti-H-Y TCR transgenic CD8+ T cells, anergized in male nu/nu recipients by a TCR-CD8 down-regulation mechanism (37), regained in vivo proliferative capacity when parked for 8 weeks in syngenic nu/nu females (38). (iii) Inhibition of the conventional helper CD4+ T cell response to viral antigen in antigen transgenic mice was also rapidly abrogated after a 1-week incubation of their T splenocytes in their non-transgenic SCID counterparts (39). (iv) The kinetics of peripheral tolerance induced by staphylococcal enterotoxin B superantigen in CD4+ Vβ8+ T cells also showed an in vivo anergy reversion 12 weeks after the end of tolerogen treatment (40). Only the tolerance of CD4+ T cells to Mls-mismatched bone-marrow grafts, involving peripheral anergy, was not reversed in hosts; however, in this case, the established grafts acted plausibly as a continuous source of tolerance (41).

It is highly unlikely that the recruitment of T lymphocytes that would actively maintain tolerance in T cells (23) could be involved in our system. Indeed, it is difficult to conceive that such tolerance-maintaining T cells would operate endlessly in IgG2aβ pn/Tx Ighhα mice or at the level of long-term parked tolerant T cells, but that they would cease to function in the euthymic IgG2aβ pn counterparts. This hypothesis would suppose that such actively maintaining tolerance T cells would only act on anti-IgG2aβ T cells exposed to high levels of tolerogen but not on newly emerging anti-IgG2aβ T cells. In in vivo parking experiments, contaminating IgG2aβ-primed antigen-presenting cells could possibly be co-transferred, with tolerant Ighhα T cells, into nu/nu mice. However, the hypothesis of definitive tolerance persistence by the action of these possible antigen-presenting cells can be discarded. Indeed, these latter might be capable of maintaining tolerance in IgG2aβ pn Ighhα donors. Conversely, the total but temporary absence of an anti-IgG2aβ T cell response could be the effect of transient exhaustion of anti-IgG2aβ T cells (42–44). Indeed, it is quite possible, if the anti-IgG2aβ T cells were able to leave the thymus, that the almost simultaneous activation and differentiation of all available anti-IgG2aβ T cells into suppression inducers or effectors, upon repeated IgG2aβ administrations, were followed by their total simultaneous disappearance by activation-induced cell death. Tolerance breakdown and re-establishment of the suppression-induction capacity would then be always possible by a still dynamic thymic output after tolerogen clearance.

In Ighhβ mice, transgenic for a certain MHC class II-restricted anti-IgG2aβ TCR, CD4+CD8+ double-positive thymocytes are not deleted, the mature CD4+ transgenic T cells do not become tolerant at the periphery and the IgG2aβ is subjected to an apparent CD8- independent suppression (45). This example moves far from the physiological situation, i.e. CB20 and C57BL/6 Ighhα mice which are naturally tolerant to their self IgG2αβ and express substantial levels of this serum Ig allotype. On the other hand, the suppression-
mechanism of T cell tolerance to IgG2ab

1059

maintaining T cell effectors of the intrinsic anti-IgG2ab T cell activity of the normal Igh strain mice that we study express the CD8 phenotype and are MHC class I-restricted. Moreover, our investigations showed that perinatal exposure of natural anti-IgG2ab T cells to this Ig allotype results in the establishment of profound specific tolerance to IgG2ab due, according to the most probable interpretation, to the physical elimination of these T cells. Certainly, it is necessary, as recently underlined (46), to be very cautious with conclusions established from experiments carried out with TCRαβ transgenic mice expressing the relevant antigen because of certain unusual IgG2ab CD4αβ T cell clones, e.g. of low affinity (49) or specific to non-dominant Epitopes (50), could escape tolerance induction or be selectively maintained during amplification of T cell subsets from sensitized Igha or Ighb mice. Nevertheless, a minority of specific T clones, e.g. of low affinity (49) or specific to non-dominant Epitopes (50), could escape tolerance induction or be subjected to reversible tolerance.

The reasons for the selection and maintenance of this constitutive anti-IgG2ab T cell activity during the evolution of various Igh mouse strains remain unclear. We previously demonstrated that the basic normal anti-IgG2ab T cell activity—which very probably reflects a relatively high frequency of anti-IgG2ab T cells in the absence of any preliminary stimulation—was not the result of peptide-dependent positive selection (reviewed in 51) of these T lymphocytes by elements encoded by Igha genes. In addition, even in Ighab and Ighb mice, the absence of IgG2ab during the perinatal period is sufficient for the spontaneous emergence of autoimmune anti-IgG2ab T cells. The implication, in this system, of intrinsic anti-IgG2ab CD4+ and CD8+ T cells with high potential to generate allo- or auto-reactivity (in Igha or Ighb mice respectively), with well characterized T-B cell interactions, provides an in vivo situation to explore natural T cell tolerance or that induced by soluble or membrane-bound form of an Ig. We are now attempting to determine whether both anti-IgG2ab CD4+ and CD8+ T subsets, required for suppression induction, are subjected to this tolerance.

Acknowledgements
This work was supported by grants from the Institut Pasteur (3540), the Centre National de la Recherche Scientifique (URA 1961) and the Association Française Contre les Myopathies. We gratefully acknowledge Christèle Sellier for excellent technical assistance, Pascal Dardenne for animal care and for performing all the bleedings, Andree Goyat for serum preparations, and Sylvana Thepaut for excellent secretarial assistance. We gratefully thank Janet Jacobson for correcting the English version of this paper.

Abbreviations

IgG2a b

Igha mice perinatally treated with soluble IgG2ab

IgG2ab p/Tx

IgG2a b pn thymectomized mice

Tseps

nylon-wool non-adherent T splenocytes from Igha mice primed against IgG2ab

Tx mice

thymectomized mice

References


5 Benaroch, P. and Bordenave, G. 1989. T cell-induced Ig allotypic suppression in mice. II. Both CD4+CD8+ and CD4+CD8– T cell subsets from sensitized Ighb mice are required to induce suppression of Igh-1b allotype expression. J. Immunol. 142:171.


9 Majlessi, L. and Bordenave, G. 1997. The T-B cell interaction involved in induction of the mouse IgG2a suppression is major
1060 Mechanism of T cell tolerance to IgG2aβ


