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Review

Mechanisms of Mouse T Lymphocyte-Induced Suppression of the IgG_{2a}^{b} Allotype and T Lymphocyte Tolerance to IgG_{2a}^{b}

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Abstract. In mice of the Igh^{a} immunoglobulin allotypic haplotype we found, the presence of T lymphocytes with an inherent inhibitory activity against the expression of the IgG_{2a}^{b} allotype (IgG_{2a} of the Igh^{b} immunoglobulin allotypic haplotype). This constitutive anti-IgG_{2a}^{b} T lymphocyte activity can be enhanced *in vivo* by what we called "sensitization", which usually consists of one or two intravenous injections of B splenocytes from Igh^{b} congenic mice. When injected at birth, the resulting anti-IgG_{2a}^{b} T splenocytes induce, with 100% success, total, specific and chronic (but experimentally reversible) suppression of IgG_{2a}^{b} in $Igh^{a/b}$ F_1 hybrid mice prepared by mating Igh congenic mice. Even if restricted to IgG_{2a}^{b} expression, this experimental model, which deals with an unambiguous case of T cell-mediated down-regulation of immunoglobulin production, provides a clear and powerful tool to dissect finely the behavior of the partners (T and B lymphocytes) intervening in regulation within the immune system. For example, we observed that CD4 T lymphocytes were necessary to obtain full recruitment of anti-IgG_{2a}^{b} CD8 T lymphocytes during the sensitization, that suppression induction in anti-IgG_{2a}^{b} T splenocytes of newborn recipients required cooperation between CD4 and CD8 T lymphocytes, and that CD8 T lymphocytes were essential for suppression maintenance. We showed that this suppression was not characterized by an accumulation of B lymphocytes containing the allotype they could not secrete or $C\gamma_{2a}^{b}$ mRNA they could not translate. The recipient's immune system was not involved in the suppession maintenance; this was done by donor T lymphocytes, which ensured the chronicity of IgG_{2a}^{b} suppression throughout the recipient's life. We demonstrated that the mechanism of this suppression implied an MHC-restricted presentation by target B lymphocytes of $C\gamma_{2a}^{b}$ peptides to the T cell receptor (TCR) of anti-IgG_{2a}^{b} T lymphocytes. Notwithstanding the requirement of a CD4-CD8 T lymphocyte cooperation during the induction phase, we functionally determined that the suppression induction implicated an MHC class I-, but not class II-restricted interaction. We also demonstrated the existence *in vivo* of alternative or concomitant use of perforine- and Fas-mediated cytotoxicity pathways in this T cell-induced IgG_{2a}^{b} suppression. Thus this suppression did not imply silencing IgG_{2a}^{b} production, but B lymphocyte destruction by CD8 T lymphocytes. Always using our suppression model, we demonstrated that an agonistic anti-CD40 treatment helps in recruiting CD8 cytotoxic T lymphocytes, involved in immune regulatory functions and that CD40 expression on Igh^{b} B lymphocytes confronted with CD8 T lymphocyte effectors only operating via the Fas pathway was involved in the total suppression of IgG_{2a}^{b} expression. The selection and maintenance of such normal T cell activity against the IgG_{2a}^{b} allotype in mice of different genetic backgrounds remain somewhat enigmatic. Indeed, we did not observe any similar activity against other immunoglobulin allotypes or isotypes. The intestinal flora had no influence on the emergence of this anti-IgG_{2a}^{b} T lymphocyte

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activity, as it was untouched in germ-free Igh^a mice when compared with normal Igh^a mice. More recently, this model offered an opportunity to study problems pertaining to immune tolerance. For instance, we showed that the genetic elements involved in the building of anti-$IgG_{2a}{}^b$ TCR were available in Igh^a and Igh^b mice of different genetic backgrounds, but that somatic constraints, namely the perinatal presence of $IgG_{2a}{}^b$, effectively prevented their acquisition, while its absence led to their spontaneous emergence. Consequently, we were able to induce anti-$IgG_{2a}{}^b$ T lymphocytes into a tolerance state by injecting Igh^a mice with soluble $IgG_{2a}{}^b$ during the perinatal period. However, the full T lymphocyte tolerance obtained in this manner was not definitively acquired, as it had reversed spontaneously when investigated 3 to 6 months after the end of tolerogen treatment, even when this treatment had been prolonged from the perinatal period to 9 months of age. The mechanisms (induction and reversion) of this tolerance involves the physical elimination or the irreversible inactivation of the natural anti--$IgG_{2a}{}^b$ T lymphocyte clones and their resurgence, from bone-marrow precursors, as long as the thymus remains operational, but not the establishment of a reversible, functional unresponsiveness (anergy) or an active, cell--mediated inhibition of anti-$IgG_{2a}{}^b$ T clones. We attempted to elucidate, in Igh^b mice, whether the natural T lymphocyte unresponsiveness to $IgG_{2a}{}^b$ involved a central tolerance mechanism and to identify the type of tolerogen implicated in this tolerogenesis. The experiments principally showed that this natural T lymphocyte tolerance to $IgG_{2a}{}^b$ was mediated by a thymic mechanism; that the capacity to induce it was gradually acquired by Igh^b thymuses and was most probably due to potentially $IgG_{2a}{}^b$-producing/presenting cells, progressively colonizing the developing thymus; and that a significantly decreased postnatal $C\gamma_{2a}{}^b$ gene transcription correlated with the emergence of anti-$IgG_{2a}{}^b$ T lymphocytes in Igh$^{a/b}$ F_1 (postnatally deprived of their B lymphocyte compartment), which subjected them to autoimmune $IgG_{2a}{}^b$-allotype suppression.

Key words: T lymphocytes; $IgG_{2a}{}^b$ allotype; suppression; tolerance; cytotoxicity; down-regulation of Ig production.

Introduction

A few years after OUDIN[32–35] discovered, using rabbit Ig, what he termed the allotypy of proteins (allotypy reflecting, at the immunogenic level, the allelism of protein structural genes), DRAY[11], also using rabbit Ig, made the first observation of "allotype suppression". The perinatal exposure of heterozygous rabbits to antibodies directed against paternally inherited Ig allotypes could lead to a chronic abrogation of their expression. These early experiments paved the way for all the subsequent manipulations of the immune system with antibodies.

In mice, the only known allotype suppression was directed against the IgG_{2a} of the Igh^b allotypic haplotype ($IgG_{2a}{}^b$). HERZENBERG et al. [14] obtained it by injecting anti-$IgG_{2a}{}^b$ antibodies into newborn Igh$^{a/b}$ (BALB/c × SJL) hybrids, whereas BOSMA'S team[8] injected splenocytes from Igh$^{a/a}$ (BALB/c) mice immunized against $IgG_{2a}{}^b$ into sublethally irradiated Igh$^{b/b}$ congenic (CB17) homozygotes. HERZENBERG'S group[12, 13] showed that the chronicity of the antibody-induced allotype suppression was exerted by T cells and advanced the idea that its mechanism was an inhibition of the helper action of T lymphocytes. This scheme, however, opposed the fact that this suppression was transferable to syngenic nude mice where thymus-independent $IgG_{2a}{}^b$ production was inhibited[15]. Except for an allotypic variant of the λ_1 light chain, all mouse Ig allotypic specificities are located on the heavy-chain constant domains of the different Ig classes and subclasses.

Starting from the work of NISONOFF'S group[10, 39], who successfully obtained idiotype suppression in mice with T cells "educated" against the given idiotype, we tried to establish a system leading to allotype suppression by means of T cells with a deliberately induced anti-allotype activity. Using pairs of Igh congenic mice (BALB/c (Igh^a, H-2^d)/CB20 (Igh^b, H-2^d)) and (BC8 (Igh^a, H-2^b)/C57BL/6 (Igh^b, H-2^b)) and their Igh$^{a/b}$ heterozygous offspring, we studied, in the latter, the effects of the transfer of T splenocytes from a parental strain on the expression of the different Ig allotypes inherited from the other parental strain. Therefore, we attempted, in histocompatible hybrids, to specifically train T lymphocytes from an Igh^a or Igh^b parental strain to react against Ig allotypic determinants genetically transmitted by the Igh^b or Igh^a strain, respectively. In the absence of any preliminary stimulation or immunization, we observed that T splenocytes from normal Igh^a mice (referred to as Tnor) with different genetic backgrounds, i. e. BALB/c (H-2^d), BC8 (H-2^b), possessed an inherent inhibitory activity against the expression of the $IgG_{2a}{}^b$ allotype[1]. This constitutive anti-$IgG_{2a}{}^b$

T lymphocyte activity of normal Igh^a mice can be enhanced *in vivo* by one or two intravenous injections of B splenocytes from an Igh^b congenic strain, $IgG_{2a}{}^b$-producing histocompatible plasmocytoma or hybridoma cells, or autologous splenocytes covalently coated with $IgG_{2a}{}^b$. Such an operation is termed "sensitization" and the resulting mice and T lymphocytes will be referred to as sensitized mice, and Tsens or T2sens, according to the number of sensitizations. Four times fewer Tsens than Tnor (1×10^7 versus 4×10^7) are sufficient to induce total, specific and chronic (but experimentally reversible) suppression of $IgG_{2a}{}^b$ expression in 100% of the $Igh^{a/b}$ recipients as early as 6 weeks of age versus around 70% of $Igh^{a/b}$ recipients subjected to suppression at 6 months of age with Tnor[2]. The suppression can be transferred into histocompatible $Igh^{a/b}$ newborns by injection of T splenocytes from mice subjected to suppression. Unlike Tnor, T2sens are also able to induce $IgG_{2a}{}^b$ suppression in $Igh^{b/b}$ congenic mice. Because the suppression is strictly restricted to the $IgG_{2a}{}^b$ allotype, it can be thought that its mechanism operates downstream from the allelic exclusion and isotypic switch leading to $IgG_{2a}{}^b$-producing plasmocytes. It must be emphasized that anti-allotype antibodies were never detected in either $Igh^{a/a}$-sensitized mice or in $Igh^{a/b}$ or $Igh^{b/b}$ Tsens recipients.

One of the advantages of our model is that it deals with an unambiguous case of T cell-mediated down--regulation of Ig production. With this *in vivo* experimental system, we are thus able to interrupt or provoke, at will, the production of large amounts of Ig. Consequently, it has enabled us to dissect finely the behavior of T and B lymphocyte partners intervening in a negative regulation of the immune system.

More recently it has offered an opportunity to study problems pertaining to immune tolerance. We developed two models to try to explore this new aspect of allotype suppression more thoroughly. As mentioned above, the first makes use of postnatal transfer of sensitized $Igh^{a/a}$ T splenocytes into histocompatible $Igh^{a/b}$ or $Igh^{b/b}$ newborns in which serum allotype production was sequentially monitored. The second consists of the cotransfer of histocompatible $Igh^{b/b}$ B and/or $Igh^{a/a}$ T cells of wild-type or diverse "knocked-out" origins into immunodeficient mice "knocked-out" for the recombination activation gene 2 *(RAG-2* gene) in which circulating allotypes were also studied. This latter model has the advantage that the implantation of transferred wild-type or different mutant B or T cells can be directly verified by FACS analysis of peripheral blood lymphocytes from RAG-$2^{-/-}$ hosts, which are totally devoid of endogenous mature B and T cells.

$IgG_{2a}{}^b$ Suppression

Main characteristics at the T cell level

Three phases can be distinguished during this suppression of $IgG_{2a}{}^b$ expression: 1) the amplification of the intrinsic anti-$IgG_{2a}{}^b$ activity of T lymphocytes in Igh^a mice, 2) the induction of suppression with these T cells, and 3) the suppression chronicity in $Igh^{a/b}$ or $Igh^{b/b}$ recipients. When Igh^a mice were deprived, before and after amplification of their constitutive anti-$IgG_{2a}{}^b$ T lymphocyte activity, of either CD4 or CD8 T lymphocytes, the resulting T splenocyte population did not have all the anti-$IgG_{2a}{}^b$ capacities of the T lymphocytes elicited in the presence of both T subpopulations. Particularly, CD4 T lymphocytes were essential to obtain full recruitment of anti-$IgG_{2a}{}^b$ CD8 T lymphocytes during sensitization[25]. We also established that suppression induction in the Tsens newborn recipients required cooperation between CD4 and CD8 T lymphocytes. The suppression-induction capacity was drastically diminished when the Tsens preparation was subjected to *in vitro* cytotoxic anti-CD4 or anti-CD8 treatment and was fully restored with a mixture of these two Tsens populations obtained by such *in vitro* treatments[3].

Wondering whether only one or both of these T subpopulations were specific to $IgG_{2a}{}^b$, we examined whether a functional complementarity could exist in the mixture of Tsens CD4 + Tnor CD8 or Tnor CD4 + Tsens CD8. The transfer of these preparations into $Igh^{a/b}$ newborns showed that the suppression induction capacities of these cell mixtures were equal to that of Tnor. Consequently, cooperation between the T populations during the suppression induction phase required both CD4 and CD8 T lymphocytes, which had to possess the anti-$IgG_{2a}{}^b$ specificity[25].

We then succeeded in attempting to abrogate the chronicity of this suppression. Injections of a cytotoxic anti-CD8 monoclonal antibody (mAb) into $IgG_{2a}{}^b$-suppressed $Igh^{b/b}$ homozygotes quickly but transiently reversed the suppression, while injections of equivalent amounts of cytotoxic anti-CD4 mAb remained without effect[5]. The corollaries of this observation are that: 1) CD8 T lymphocytes are essential for suppression maintenance, and 2) this suppression does not reflect a clonal deletion of the B lymphocytes able to produce $IgG_{2a}{}^b$; consequently, it does not involve the definitive elimination of the B precursors of IgG2a-producing lymphocytes which, in contrast, might exhibit a permanent tendency to reappear. This latter situation would favor the continuous stimulation of the suppression maintaining CD8 T lymphocytes, which then would

bypass the helper factors provided by CD4 T lymphocytes that are indispensable during the suppression induction phase[5].

This suppression could also be transiently abolished *in vivo* not only by CD8 T lymphocyte depletion but by the reinforcement of CD4 T lymphocytes, which help Ig production, as well. Indeed, $IgG_{2a}{}^{b}$-suppressed $Igh^{b/b}$ homozygotes presented a temporary abrogation of their suppression when the hosts were infected with *Plasmodium chabaudi* or *Trypanosoma cruzi* parasites, which induce CD4-dependent polyclonal T lymphocyte activation. When the $IgG_{2a}{}^{b}$-suppressed mice were concomitantly subjected to anti-CD4 treatment and parasite infection, the polyclonal activity was drastically lower and the suppression remained complete[4].

Main characteristics at the B cell level

The use of an RNAse protection assay, in which a specific antisense probe was hybridized to the $C\gamma_{2a}{}^{b}$ allele, enabled us to show that the $C\gamma_{2a}{}^{b}$-mRNA level was markedly lower (150 to 400 times) in $IgG_{2a}{}^{b}$-suppressed $Igh^{b/b}$ mice than in age-matched $Igh^{b/b}$ controls. Therefore, the suppression did not represent a complete inhibition of $C\gamma_{2a}{}^{b}$-gene transcription. We also showed that it is not characterized by an accumulation of B lymphocytes containing the allotype they could not secrete or, as we saw before, $C\gamma_{2a}{}^{b}$ mRNA they could not translate[6].

Origin of the CD8 T lymphocytes responsible for this suppression

To investigate the origin, either donor or host, of the T lymphocytes responsible for maintaining suppression, we used, on the C57BL/6 background, mice doubly congenic, at the *Igh* and *Thy-1* loci. The donor cells were Igh^{a}, H-2^{b}, Thy-1.2 while the recipients were Igh^{b}, H-2^{b}, Thy-1.1. FACS analysis indicated that, at adulthood, the donor-Thy-1.2 T lymphocytes represented 3 to 5% and 5 to 10%, respectively, of total splenocytes and lymph node cells in the Thy-1.1 hosts, whose T lymphocyte compartment was therefore a "Thy-1.1 + Thy-1.2 chimera". Splenocytes from these 6- or 12-month-old chimeras were subjected to *in vitro* anti-Thy-1.1 or anti-Thy-1.2 cytotoxic treatment before being transferred into histocompatible $Igh^{a/b}$ newborns for suppression induction assays. We observed that only the donor-Thy-1.2 T lymphocytes were able to transfer the $IgG_{2a}{}^{b}$ suppression and that the host-Thy-1.1 T lymphocytes were totally devoid of such capacity. This observation proved that the recipient's immune system was not involved in suppression maintenance and that the donor-Thy-1.2 T lymphocytes, by their effective and permanent engraftment into histocompatible hosts, ensured the chronicity of $IgG_{2a}{}^{b}$ suppression throughout the recipient's life[24].

Cellular mechanism of this suppression

Combining the algorithm of the peptidic self-model[9, 17, 18] with the comparison of the protein sequences of the $C\gamma_{2a}{}^{a}$ and $C\gamma_{2a}{}^{b}$ regions, where allotopes are located, we selected four "rare" peptides (10 to 20 amino acids long) from the $C\gamma_{2a}{}^{b}$ chain, suspected to be immunogenic for Igh^{a}, H-2^{d} or H-2^{b} T lymphocytes. We compared the abilities of these peptides to amplify the anti-$IgG_{2a}{}^{b}$ T lymphocyte activity of Igh^{a} mice with that of the entire $IgG_{2a}{}^{b}$ allotype. These experiments showed that, in Igh^{a}, H-2^{d} (BALB/c) mice, one peptide from the CH3 domain was as effective as the intact $IgG_{2a}{}^{b}$ allotype at sensitizing the anti-$IgG_{2a}{}^{b}$ T lymphocytes, while the other $C\gamma_{2a}{}^{b}$ peptides had no amplifier effect. In Igh^{a}, H-2^{b} (BC8) mice, another peptide, this time from the hinge region, was the only one able to obtain 100% sensitization efficacy, while the other $C\gamma_{2a}{}^{b}$ peptides were totally inactive. These data demonstrated that the mechanism of this suppression involved a major histocompatibility complex (MHC)-restricted presentation of $C\gamma_{2a}{}^{b}$ peptides by the target B lymphocytes to the T cell receptor (TCR) of anti-$IgG_{2a}{}^{b}$ T lymphocytes. In addition, despite the same macroscopic characteristics of this suppression in H-2^{d} and H-2^{b} mice, namely the T cell-mediated complete inhibition of $IgG_{2a}{}^{b}$ production, the fine specificity of the anti-$IgG_{2a}{}^{b}$ TCR varied as a function of the MHC haplotype of the target B lymphocytes[27].

MHC restriction of anti-$IgG_{2a}{}^{b}$ T cells

To address this problem, we chose to use Igh^{b} C57BL/6 mice "knocked-out" for MHC class I (β_2-microglobulin: $\beta_{2m}{}^{-/-}$) or class II (I-A$\beta^{-/-}$) molecules. These mice can adoptively receive Tsens from the Igh^{a}, H-2^{b} BC8 congenic strain for investigations of suppression induction. Since we have shown that the T cell compartment of Igh^{b} hosts does not play any active role in the suppression mechanism, the absence of CD4 or CD8 T lymphocytes, respectively, in I-A$\beta^{-/-}$ or $\beta_{2m}{}^{-/-}$ recipients does not influence the suppression exerted on their $IgG_{2a}{}^{b+}$ B lymphocytes. We demonstrated that the Igh^{a}, H-2^{b} anti-$IgG_{2a}{}^{b}$ T lymphocytes transferred into Igh^{b}, H-2^{b} I-A$\beta^{-/-}$ mice carried out the suppression process normally, while in Igh^{b}, H-2^{b} $\beta_{2m}{}^{-/-}$ recipients, their

suppression induction capacity was significantly inhibited (around 70% of IgG_{2a}^{b}-expressing mice, while implantation, approximately 12% of both CD4 and CD8 donor T lymphocytes, was identical in IgG_{2a}^{b}-suppressed and -unsuppressed recipients) [20]. Consequently, we showed, based on function evaluation, that the suppression induction implicated an MHC class I-, but not class II-restricted interaction, despite the requirement of a CD4-CD8 T lymphocyte cooperation during the induction phase. This MHC class I-restriction was in good agreement with our previous designation of CD8 T lymphocytes as essential for suppression maintenance[20].

In addition to class I and class II molecules, the mouse MHC codes for other structures, named class Ib, which present striking conformational and sequential homologies with classical MHC class I molecules and associate with β_{2m}. Among them, the Qa-1b molecule possesses a functional pocket for peptide presentation. The potential role of Qa-1b was suggested in two negative immunoregulation models involving, like ours, CD8 T lymphocytes. The fact that most $\beta_{2m}^{-/-}$ mice recipients of Tsens are unsuppressed led us to examine the possible role of the Qa-1b molecule in the MHC restriction of the T-B interaction implied in the IgG_{2a}^{b} suppression. Reconstitution of RAG-$2^{-/-}$ mice with Igh^{a} T lymphocytes and Igh^{b} Qa-1a B lymphocytes (while wild-type mice are Qa-1b) induced total suppression, thereby showing that Qa-1b was not involved in this regulation (our unpublished results).

Does the inhibition of IgG_{2a}^{b} expression depend on suppression or cytotoxicity?

We sought to establish whether the IgG_{2a}^{b}-suppression effectors, namely MHC class I-restricted CD8 T lymphocytes, cytolysed their IgG_{2a}^{b+} targets or only silenced IgG_{2a}^{b} production. To do so, we looked at the two principal mechanisms of cell death for T cell-mediated cytotoxicity, i.e., the pore-forming protein (Pfp)- and Fas-dependent pathways. Because wild-type Igh^{a} Tsens failed to engraft in Igh^{b} $Fas^{-/-}$ congenic recipients, we used, for this investigation, the system of RAG-$2^{-/-}$ mice reconstituted with histocompatible T lymphocytes from Igh^{a} wild-type mice ($Pfp^{+/+}$) or from *Pfp*-gene "knocked-out" mice ($Pfp^{-/-}$ mice) and B lymphocytes from wild-type mice ($Fas^{+/+}$) or from Lpr/Lpr (*Lpr* for lymphoproliferation mutation or $Fas^{-/-}$) mice.

We first showed that B lymphocytes from $Fas^{+/+}$ or $Fas^{-/-}$ Igh^{b} mice were subjected to the full and specific IgG_{2a}^{b} suppression in the presence of wild-type $Pfp^{+/+}$ Igh^{a} Tsens. Thus, under the conditions of blockage of only the Fas-dependent cytotoxicity pathway, it was quite possible to induce full suppression against IgG_{2a}^{b+} B lymphocytes. Second, we observed that T lymphocytes from $Pfp^{+/+}$ or $Pfp^{-/-}$ Igh^{a} mice were able to induce total and specific IgG_{2a}^{b} suppression in the presence of B lymphocytes from wild-type $Fas^{+/+}$ Igh^{b} mice. Pertinently, we demonstrated that, under simultaneous blockage of Pfp- and Fas-dependent cytotoxicity pathways, namely when $Pfp^{-/-}$ Igh^{a} T lymphocytes were confronted with $Fas^{-/-}$ Igh^{b} B lymphocytes, the IgG_{2a}^{b}--suppression induction was totally inhibited.

Taken together, our results show the existence *in vivo* of alternative or concomitant use of Pfp- and Fas--mediated cytotoxicity pathways in this T cell-induced IgG_{2a}^{b} suppression[21], namely, in T cell-mediated down--regulation of Ig production. The involvement of both Pfp- and Fas cell-death factors in this model strongly suggests that apoptosis constitutes the mechanism of this T lymphocyte-mediated IgG_{2a}^{b} suppression. As each of these pathways is sufficient to ensure full suppression, this duplication could represent a kind of safety valve to prevent hypothetical disorders possibly arising in the absence of such T cell function. Another obvious consequence is that this IgG_{2a}^{b} suppression does not imply silencing IgG_{2a}^{b} production, but B lymphocyte destruction by CD8 T lymphocytes via Pfp- and/or Fas-dependent cytotoxicity.

The oncoprotein BCL-2 possesses an intracellular anti-apoptosis regulatory property. In our system we investigated the possible impact of constitutive BCL-2 overexpression on Fas-mediated B cell death. In RAG--$2^{-/-}$ mice reconstituted with Igh^{b} B lymphocytes transgenic for human BCL-2 (there is no species barrier for BCL-2) and $Pfp^{-/-}$ Igh^{a} T lymphocytes (which can use only the Fas-mediated death pathway), the BCL-2--transgenic B lymphocytes were subjected to total IgG_{2a}^{b} suppression. Despite high intracellular expression of functional transgenic BCL-2 and no up-regulation of the main BCL-2 inhibitors (BCL-xs, BAX), IgG_{2a}^{b} suppression continued to be observed under conditions in which the Fas-dependent cytotoxicity pathway was exclusively exerted. Consequently, in these mature B lymphocytes, the negative regulation of IgG_{2a}^{b} production implies a pathway in which the BCL-2 oncoprotein has no protective effect against Fas-mediated cytotoxicity[22].

The role of CD40 in the suppression of IgG_{2a}^{b} expression

According to the model of "sequential cellular interactions" [7, 36, 37], designed to try to explain the CD4-

-dependent generation of CD8 cytotoxic T lymphocytes (CTL), the CD4 T lymphocyte help for CD8 T lymphocyte responses is essentially mediated by the intermediary of antigen-presenting cells (notably dendritic cells: DC) carrying both MHC class I and class II components able to present antigen-derived peptides to CD8 and CD4 T lymphocytes, respectively. The engagement of CD40 on DC with its ligand (CD40L) on activated CD4 T lymphocytes activate the DC, which, in turn, acquire the capacity to stimulate CD8 CTL directed against the same antigenic determinant. For example, in the absence of CD4 T lymphocytes, their help can be restored by the action of agonistic anti-CD40 Ab. Since the suppression of IgG_{2a}^{b} expression provides a good example of CD4 T lymphocyte-dependent generation of CD8 CTL, we wondered whether CD4 T lymphocyte-depleted Igh^{a} mice, subjected to *in vivo* agonistic anti-CD40 treatment and, of course, sensitized against Igh^{b} congenic B lymphocytes, would be able to recruit regulatory CD8 T lymphocytes with the potential to inhibit IgG_{2a}^{b} production in $Igh^{a/b}$ recipients. We observed that this was indeed the case but, despite their marked ability to negatively regulate IgG_{2a}^{b} production, the anti-IgG_{2a}^{b} T lymphocytes recruited by CD40 stimulation in CD4 T lymphocyte-depleted Igh^{a} mice failed to induce full suppression in all the $Igh^{a/b}$ recipients, as if they were less effective than anti-IgG_{2a}^{b} CD8 T lymphocytes normally obtained in the presence of CD4 T lymphocytes. This incomplete replacement of CD4 T lymphocyte help by *in vivo* CD40 triggering could reflect the need for a parallel CD40-independent DC stimulation or direct CD4-CD8 T lymphocyte communication for the priming of CD8 CTL[23]. Nevertheless, our data provide evidence that agonistic anti-CD40 treatment helps recruit CD8 CTL implicated in immune regulatory functions.

Alternatively, we also investigated the possible role of CD40 on the suppression induction phase. Because Fas is not expressed on the surface of mature B lymphocytes, its up-regulation, particularly via CD40 stimulation, would constitute a prerequisite for induction and maintenance of IgG_{2a}^{b} suppression by means of the Fas-mediated pathway. Thus, our model also provides the opportunity to investigate the possible involvement of CD40 expression on Igh^{b} B lymphocytes when the Igh^{a} CD8 T lymphocyte effectors operate exclusively via the Fas pathway. By implanting appropriate Igh^{a} T and Igh^{b} B lymphocytes into histocompatible RAG-$2^{-/-}$ recipients, we were able to demonstrate that $Pfp^{-/-}$ Igh^{a} T lymphocytes (exclusively operating through the Fas-mediated cytotoxicity pathway) failed to establish full suppression against $CD40^{-/-}$ Igh^{b} B lymphocytes, while they were able to totally induce it against $CD40^{+/+}$ Igh^{b} B lymphocytes. We can easily imagine the existence of two kinds of IgG_{2a}^{b}-producing B lymphocytes: 1) a highly represented population, readily susceptible to Fas-induced suppression, which up-regulates Fas through a CD40-independent mechanism, and 2) a weakly represented population, chronically escaping Fas-induced suppression in the absence of CD40-mediated Fas up-regulation. Alternatively, when CD40-dependent Fas up-regulation is impossible, Fas-mediated cytolysis of B cells recently committed to IgG_{2a}^{b} production would be somewhat delayed before they become vulnerable to CD40-independent Fas-mediated death. During this latent period, minute amounts of IgG_{2a}^{b} could be secreted per cell and would lead to the presence of weak, but detectable, serum IgG_{2a}^{b} [23].

We also observed that transfer of $CD40^{+/+}$ or, even more so, $CD40^{-/-}$ mature peripheral B lymphocytes into an RAG-$2^{-/-}$ environment led to enhanced switch recombination to IgG_{2a}^{b}. For $CD40^{-/-}$ B lymphocytes, the order of magnitude of frequency and absolute number of IgG_{2a}^{b}-producing cells were increased around 500 times when transferred into the RAG-$2^{-/-}$ environment[23].

The Restriction of this Suppression to the IgG_{2a}^{b} Allotype

The reasons for the suppression-induction restriction to the IgG_{2a}^{b} allotype, and the selection and the maintenance of such T lymphocyte function during mouse evolution, have not been elucidated. We formulated two hypotheses. A possible explanation could be a regulation remnant existing in Igh^{a} mice from a time when the two genes, $C\gamma_{2a}^{a}$ and $C\gamma_{2a}^{b}$, were tandemly organized, as is still observed in feral mice[16, 19, 31, 38]. This means that if the products of these two genes have a "present" of allotypes in laboratory mice, they have a "past" of isotypes in feral mice. Another possibility could be a fortuitous cross-reactivity between a still unknown T lymphocyte function and an Ig allotype, i.e. IgG_{2a}^{b}.

So far, our investigations in these directions have been unsuccessful. For instance, using the technique of appropriate allotype, covalently coated autologous splenocytes or Igh^{a} mice "knocked-out" for cell-surface IgM (which are totally agammaglobulinemic and, thereby, possess a T cell compartment not rendered tolerant to Ig), we were unable to detect, in Igh^{a} and Igh^{b} mice, a T lymphocyte activity other than the one

directed against IgG_{2a}^b. We did not succeed in amplifying the anti-IgG_{2a}^b T lymphocyte activity of Igh^a mice either with the immunodominant peptides of influenza virus M protein or HBs protein, respectively showing 5/8 and 4/8 amino acid-sequence homologies with the active core of $C\gamma_{2a}^b$-derived peptides, which are effective sensitizers in Igh^a, H-2^d and H-2^b mice, respectively. Moreover, despite a 6/8 amino acid-sequence homology between the core of the $C\gamma_{2a}^b$-derived peptide active in Igh^a, H-2^d mice and a peptide from the IA heavy-chain variable region subgroup (Igh V-IA), we did not find any difference in the expression (measured at the mRNA level) of this Igh V-IA in normal or sensitized Igh^a H-2^d mice, in normal or IgG_{2a}^b-suppressed Igh^b or $Igh^{a/b}$, H-2^d mice, or in normal Igh^a or Igh^b, H-2^d mice. Thus, the absence, the presence or the amplification of anti-IgG_{2a}^b T lymphocytes has no repercussion on the expression of this Igh V subgroup. Finally, this anti-IgG_{2a}^b T lymphocyte activity was untouched in germ-free Igh^a mice compared with normal Igh^a mice, showing that the intestinal flora had no influence on the emergence of this anti-IgG_{2a}^b T lymphocyte activity (our unpublished results).

Tolerance

The basis for emergence or tolerization of natural T lymphocytes specific to IgG_{2a}^b during the perinatal period

The Igh^a and Igh^b congenic mice we used possess the same genetic background and, thus, the same potential TCR repertoire. Igh^b mice produce substantial levels of IgG_{2a}^b, while their Igh^a congenic counterparts exhibit a constitutive T lymphocyte activity against this Ig allotype. In Igh^a strains, an *Igh^a* gene-encoded peptide-mediated positive selection of lymphocytes endowed with this TCR could occur. Alternatively, in Igh^b and $Igh^{a/b}$ mice, these lymphocytes could be negatively selected in order to prevent autoimmunity, by clonal deletion or anergy, using elements encoded by *Igh^b* genes. To explore these hypotheses, our experimental strategy was to deprive Igh^a, $Igh^{a/b}$ and Igh^b mice of their B lymphocyte compartment during the perinatal period, when immune competence is thought to be established. At adulthood, after the complete reconstitution of their B lymphocyte compartment, the anti-IgG_{2a}^b T lymphocyte activity was investigated. We observed that Tsens from such treated Igh^a mice totally preserved their anti-IgG_{2a}^b T lymphocyte activity, which excluded the hypothesis of an Igh^a-dependent positive selection of T lymphocytes bearing anti-IgG_{2a}^b TCR. In contrast, we found that B lymphocyte-deprived Igh^b and $Igh^{a/b}$ mice could be progressively and "spontaneously" subjected to a total, chronic (but experimentally reversible) and transferable T lymphocyte-mediated IgG_{2a}^b suppression[26].

As a corollary, we showed that the presence of IgG_{2a}^b during the perinatal period was responsible for the induction of anti-IgG_{2a}^b T cell tolerization. Indeed, perinatal exposure of Igh^a mice to soluble IgG_{2a}^b resulted in the loss of the capacity of their T splenocytes to induce the suppression in $Igh^{a/b}$ recipients. However, this full T lymphocyte tolerance was not definitively acquired, as it had reversed spontaneously when investigated 3 to 6 months after the end of tolerogen treatment, even when this treatment had been prolonged from the perinatal period to 9 months of age.

Our data demonstrated that the genetic elements involved in the construction of anti-IgG_{2a}^b TCR were available in Igh^a and Igh^b mice of different genetic backgrounds (and probably in all mouse strains), but that somatic constraints, namely the perinatal presence of IgG_{2a}^b, effectively prevented their acquisition. Conversely, the absence of IgG_{2a}^b during the perinatal period led to the spontaneous emergence of anti-IgG_{2a}^b T lymphocytes. This scheme now reflects our general understanding of this IgG_{2a}^b suppression[29].

We established that tolerance was not reversed in peripheral mature Igh^a T lymphocytes, parked *in vivo* for up to 20 weeks in histocompatible Igh^a IgG_{2a}^b-free *nu/nu* mice. In addition, the spontaneous breakdown of this tolerance was effectively prevented when *de novo* T lymphocyte maturation was impeded by thymectomy at the end of tolerogen administration. Therefore, the mechanisms (induction and reversion) of this tolerance involve the physical elimination or the irreversible inactivation of the natural anti-IgG_{2a}^b T lymphocyte clones and their resurgence, from bone-marrow precursors, as long as the thymus remains functional, but not the establishment of a reversible functional unresponsiveness (anergy) or the active cell-mediated inhibition of anti-IgG_{2a}^b T clones[28]. The high frequency of anti-IgG_{2a}^b T cell resurgence as well as the expression of the basic anti-IgG_{2a}^b T lymphocyte activity in normal Igh^a mice could suggest the existence of a high rate of $V\alpha J\alpha/V\beta DJ\beta$-*TCR* gene rearrangement for this specificity. We also observed that it was not easier to lead anti-IgG_{2a}^b T lymphocytes to a tolerance state in adult Igh^a mice thymectomized before tolerogen administration than in naive adult Igh^a mice. It seems that, once in the periphery, anti-IgG_{2a}^b T lymphocytes become re-

sistant to tolerance establishment (our unpublished results).

The thymic molecular bases of the anti-IgG$_{2a}^{b}$ natural T lymphocyte tolerance

We attempted to elucidate, in Igh^{b} mice, whether the natural T lymphocyte unresponsiveness to IgG$_{2a}^{b}$ involved a central tolerance mechanism, and to identify the type of tolerogen implicated in this tolerogenesis. We first observed that $C\gamma_{2a}^{b}$ gene transcription began in Igh^{b} thymuses by 3 to 4 days after birth and remained easily detectable thereafter. To examine the functional capacity of Igh^{b} thymuses to induce T lymphocyte tolerance to IgG$_{2a}^{b}$, based on the kinetics of thymic $C\gamma_{2a}^{b}$-mRNA expression, we transplanted thymuses from Igh^{b} or Igh^{a} control donors of diverse ages into T lymphocyte-deficient, Igh^{a} Cγ_{2a}^{b}-free *nu/nu* recipients. After reconstitution of their T lymphocyte compartment, their splenocytes were transferred into histocompatible Igh$^{a/b}$ newborns in which donor T lymphocyte tolerance was reflected by the incapacity to induce full, chronic and specific IgG$_{2a}^{b}$ suppression.

In this experimental context, thymuses still not expressing IgG$_{2a}^{b}$ or already expressing IgG$_{2a}^{b}$ from, respectively, <1-day or 1-week-old Igh^{b} newborns were unable to induce tolerance to IgG$_{2a}^{b}$, but allowed the emergence of *nu/nu*-derived anti-IgG$_{2a}^{b}$ T lymphocytes. However, thymuses from 16-week-old Igh^{b} adults exhibited marked capacity to induce full and long-lasting T lymphocyte tolerance to IgG$_{2a}^{b}$.

Taken together, these experiments[30] demonstrated that: 1) the natural T lymphocyte tolerance to IgG$_{2a}^{b}$ was mediated by a thymic mechanism; 2) the presence of circulating IgG$_{2a}^{b}$ was not essential for the natural tolerance induction of IgG$_{2a}^{b}$-specific T lymphocytes; 3) the capacity to induce T lymphocyte tolerance to IgG$_{2a}^{b}$ was gradually acquired by Igh^{b} thymuses and thereby did not constitute a property of the genetically Igh^{b} thymic epithelium; and 4) the establishment of this central T lymphocyte tolerance was most probably due to potentially IgG$_{2a}^{b}$-producing/presenting cells, progressively colonizing the developing thymus (the frequency and self-renewal potential of these producing/presenting cells in adult Igh^{b} thymuses were sufficiently high to support their survival until transplant vascularization, which was probably not the case for newborn Igh^{b} thymuses). We further showed that: 5) in the Igh^{a} *nu/nu* recipients of adult Igh^{b} thymuses, 33 to 65% of T splenocytes were of Igh^{a} *nu/nu*-recipient origin, but these peripheral Igh^{a} T lymphocytes were obviously rendered tolerant to IgG$_{2a}^{b}$ during their differentiation through the adult Igh^{b} thymuses (it is relevant to note that we were unable to detect, in Igh^{a} *nu/nu* recipients of adult Igh^{b} thymuses, peripheral expression of $C\gamma_{2a}^{b}$-mRNA or IgG$_{2a}^{b}$; this observation argues against the existence, in these hosts, of a peripheral mechanism of tolerance induction to IgG$_{2a}^{b}$); 6) finally, significantly decreased postnatal $C\gamma_{2a}^{b}$-gene transcription correlated with the emergence of anti-IgG$_{2a}^{b}$ T lymphocytes in Igh$^{a/b}$ F_1 (treated during the postnatal period with anti-mouse IgM Ab and transitorily deprived of their B lymphocyte compartment), which subjected these mice to autoimmune IgG$_{2a}^{b}$-allotype suppression.

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References

1. BÉNAROCH P. and BORDENAVE G. (1987): Normal T splenocytes are able to induce the immunoglobulin allotypic suppression in F_1 hybrid mice. Eur. J. Immunol., **17**, 167–171.
2. BÉNAROCH P. and BORDENAVE G. (1988): Enhancement in Igh^{a} mouse strains of the "natural" suppressive activity of normal T splenocytes against the expression of Igh-1b allotype. I. Molecular aspects of the chronic suppression obtained. Eur. J. Immunol., **18**, 51–58.
3. BÉNAROCH P. and BORDENAVE G. (1989): T cell-induced immunoglobulin allotypic suppression in mice. II. Both CD4^{+}CD8^{-} and CD4^{-}CD8^{+} T cell subsets from sensitized Igh^{a} mice are required to induce suppression of Igh-1b allotype expression. J. Immunol., **142**, 1–7.
4. BÉNAROCH P., FALANGA P. B., SPINELLA S. and BORDENAVE G. (1992): *Plasmodium chabaudi* and *Trypanosoma cruzi* infections can reverse IgG$_{2a}^{b}$ chronic suppression in mice. Int. Immunol., **4**, 377–385.
5. BÉNAROCH P., GEORGATSOU E. and BORDENAVE G. (1988): Cellular induction of chronic allotype suppression of IgG2a in Igh$^{b/b}$ homozygous mice and its abrogation by *in vivo* treatment with anti-CD8 monoclonal antibody. J. Exp. Med., **168**, 891–904.
6. BÉNAROCH P., GEORGATSOU E. and BORDENAVE G. (1993): T cell-induced suppression of IgG$_{2a}^{b}$ expression *in vivo* leads to large reduction of Cγ_{2a}^{b} mRNA levels. J. Immunol., **150**, 858–866.
7. BENNETT S. R., CARBONE F. R., KARAMALIS F., FLAWELL R. A., MILLER J. F. and HEATH W. R. (1998): Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature, **393**, 478–480.
8. BOSMA M. J. and BOSMA G. C. (1976). Chronic suppression of

immunoglobulin allotype production in adult congenic mice. Nature, **259**, 313–315.
9. CLAVERIE J. M. and KOURILSKY P. (1986): The peptidic self model: a reassessment of the role of the major histocompatibility complex molecules in the restriction of the T-cell response. Ann. Inst. Pasteur/Immunol., **137D**, 425–442.
10. DOHI Y. and NISONOFF A. (1979): Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. J. Exp. Med., **150**, 909–918.
11. DRAY S. (1962): Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling γ-globulin allotypic specificities. Nature, **195**, 677–680.
12. HERZENBERG L. A. and HERZENBERG L. A. (1974): Short-term and chronic allotypic suppression in mice. Contemp. Top. Immunobiol., **3**, 41–57.
13. HERZENBERG L. A., CHAN E. L., RAVITCH M. M., RIBLET R. J. and HERZENBERG L. A. (1973): Active suppression of immunoglobulin allotype synthesis. III. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node, and bone marrow. J. Exp. Med., **137**, 1311–1324.
14. HERZENBERG L. A., HERZENBERG L. A., GOODLIN R. C. and RIVERA E. C. (1967): Immunoglobulin synthesis in mice. Suppression by anti-allotype antibody. J. Exp. Med., **126**, 701–713.
15. JACOBSON E. B. (1978): Adoptive transfer of allotypic-specific suppressor cells inhibits thymus-independent immunoglobulin production in syngenic athymic mice. J. Exp. Med., **148**, 607–612.
16. JOUVIN-MARCHE E., MORGADO M. G., LEGUERN C., VOEGTLE D., BONHOMME F. and CAZENAVE P. A. (1989): The mouse Igh-1a and Igh-1b H chain constant regions are derived from two distinct isotypic genes. Immunogenetics, **29**, 92–97.
17. KOURILSKY P. and CLAVERIE J. M. (1986): The peptidic self model: a hypothesis on the molecular nature of the immunological self. Ann. Inst. Pasteur/Immunol., **137D**, 3–21.
18. KOURILSKY P., CHAOUAT G., RABOURDIN-COMBE C. and CLAVERIE J. M. (1987): Working principles in the immune system implied by the "peptidic self" model. Proc. Natl. Acad. Sci. USA, **84**, 3400–3404.
19. LIEBERMAN R. and POTTER M. (1969): Crossing over between genes in the immunoglobulin heavy chain linkage group of the mouse. J. Exp. Med., **130**, 519–541.
20. MAJLESSI L. and BORDENAVE G. (1997): The T/B cell interaction involved in induction of the mouse $IgG_{2a}{}^{b}$ suppression is restricted by major histocompatibility complex class I, but not class II molecules. Eur. J. Immunol., **27**, 1346–1352.
21. MAJLESSI L. and BORDENAVE G. (1999): Evidence of alternative or concomitant use of perforin- and Fas-dependent pathways in a T cell-mediated negative regulation of Ig production. J. Immunol., **162**, 4391–4398.
22. MAJLESSI L. and BORDENAVE G. (2000): Non-overlapping Fas- and BCL-2-regulated death pathways in $IgG_{2a}{}^{b}$-producing B cells. Int. Immunol., **12**, 969–976.
23. MAJLESSI L. and BORDENAVE G. (2001): Role of CD40 in a T cell-mediated negative regulation of Ig production. J. Immunol., **166**, 841–847.
24. MAJLESSI L., BENAROCH P. and BORDENAVE G. (1992): The T cell-induced allotypic suppression. Origin of the $CD8^{+}$ T cells maintaining $IgG_{2a}{}^{b}$ suppression. J. Immunol., **149**, 1881–1888.
25. MAJLESSI L., BENAROCH P., DENOYELLE C. and BORDENAVE G. (1993): Role of $CD4^{+}$ and $CD8^{+}$ cell subsets during amplification of natural T cell activity against $IgG_{2a}{}^{b}$ in Igh^{a} mice and during induction of $IgG_{2a}{}^{b}$ allotype suppression in $Igh^{a/b}$ mice. J. Immunol., **151**, 1859–1867.
26. MAJLESSI L., MARCOS M. A. R., BENAROCH P., DENOYELLE C. and BORDENAVE G. (1994): T cell-induced Ig allotypic suppression in mice. Basis for emergence or tolerization, during the perinatal period, of natural T cells specific to the $IgG_{2a}{}^{b}$ allotype. J. Immunol., **152**, 3342–3352.
27. MAJLESSI L., ROTH C., BENAROCH P., CLAVERIE J. M., KOURILSKY P. and BORDENAVE G. (1993): Identification of T cell peptides from $C\gamma_{2a}{}^{b}$ involved in $IgG_{2a}{}^{b}$ T cell-induced allotypic suppression. Relationship with the MHC haplotype of the suppressor T cell-donor Igh^{a} mice. J. Immunol., **151**, 1263–1272.
28. MAJLESSI L., RUJITHAMKUL N., BURLEN-DEFRANOUX O. and BORDENAVE G. (1997): Breakdown of T cell tolerance to $IgG_{2a}{}^{b}$ in Igh^{a} mice by *de novo* emerging anti-$IgG_{2a}{}^{b}$ T cells and not anergy reversion. Int. Immunol., **9**, 1053–1060.
29. MAJLESSI L., RUJITHAMKUL N., SELLIER C. and BORDENAVE G. (1996): Spontaneous breakdown of T cell tolerance in the mouse $IgG_{2a}{}^{b}$-suppression model despite long-term tolerogenesis since the perinatal period. Int. Immunol., **8**, 1627–1636.
30. MAJLESSI L., SELLIER C. and BORDENAVE G. (2000): Central tolerance induction in intrinsic immunoglobulin-allotype-specific T cells. Eur. J. Immunol., **30**, 3487–3497.
31. MORGADO M. G., CAM P., GRIS-LIEBE C., CAZENAVE P. A. and JOUVIN-MARCHE E. (1989): Further evidence that BALB/c and C57BL/6 γ2a genes originate from two distinct isotypes. EMBO J., **8**, 3245–3251.
32. OUDIN J. (1956): L'allotypie de certains antigènes protéidiques du sérum. C. R. Acad. Sci. Sér. D, **242**, 2606–2608.
33. OUDIN J. (1956): Réaction de précipitation entre des sérums d'animaux de même espèce. C. R. Acad. Sci. Sér. D, **242**, 2489–2490.
34. OUDIN J. (1960): Allotypy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of seven main allotypes. J. Exp. Med., **112**, 107–124.
35. OUDIN J. (1960): Allotypy of rabbit serum proteins. II. Relationship between various allotypes: their common antigenic specificity, their distribution in a sample population. Genetic implication. J. Exp. Med., **112**, 125–142.
36. RIDGE J. P., DI R. F. and MATZINGER P. (1998): A conditioned dendritic cell can be a temporal bridge between a $CD4^{+}$ T-helper and a T-killer cell. Nature, **393**, 474–478.
37. SCHOENBERGER S. P., TOES R. E. M., VAN DER VOORT E. I. H., OFFRINGA R. and MELIEF C. J. M. (1998): T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40 L interactions. Nature, **393**, 480–483.
38. SHIMIZU A., HAMAGUCHI Y., YAOITA Y., MORIWAKI K., KONDO K. and HONJO T. (1982): Japanese wild mouse, *Mus musculus molossinus*, has duplicated immunoglobulin γ_{2a} genes. Nature, **298**, 82–84.
39. SY M. S., BACH B. L., BROWN A., NISONOFF A., BENACERRAF B. and GREENE M. I. (1979): Antigen and receptor-driven regulatory mechanisms. II. Induction of suppressor T cells with idiotype-coupled syngenic spleen cells. J. Exp. Med., **150**, 1229–1240.

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