Induction of Peripheral T Cell Tolerance by Antigen-Presenting B Cells. II. Chronic Antigen Presentation Overrules Antigen-Presenting B Cell Activation

Giorgio Raimondi, Ivan Zanoni, Stefania Citterio, Paola Ricciardi-Castagnoli and Francesca Granucci

*J Immunol* 2006; 176:4021-4028; doi: 10.4049/jimmunol.176.7.4021

http://www.jimmunol.org/content/176/7/4021

References

This article cites 30 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/176/7/4021.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Induction of Peripheral T Cell Tolerance by Antigen-Presenting B Cells. II. Chronic Antigen Presentation Overrules Antigen-Presenting B Cell Activation

Giorgio Raimondi, Ivan Zanoni, Stefania Citterio, Paola Ricciardi-Castagnoli, and Francesca Granucci

Ag presentation in the absence of danger signals and Ag persistence are the inductive processes of peripheral T cell tolerization proposed so far. Nevertheless, it has never been definitively shown that chronic Ag presentation per se can induce T cell tolerance independent of the state of activation of APCs. In the present work, we investigated whether chronic Ag presentation by either resting or activated B cells can induce tolerance of peripheral Ag-specific T cells. We show that CD4+ T cells that re-encounter the Ag for a prolonged period, presented either by resting or activated Ag-presenting B cells, become nonfunctional and lose any autoimmune reactivity. Thus, when the main APCs are B cells, the major mechanism responsible for peripheral T cell tolerization is persistent Ag exposure, independent of the B cell activation state. The Journal of Immunology, 2006, 176: 4021–4028.

The immune system of vertebrate animals has the capacity to respond to perturbations (invading pathogens, stress signals) limiting self-tissue damage. Tolerance to tissue Ags is achieved through a combination of thymic and peripheral events that eliminate or inactivate potentially dangerous T cells. Several models have been proposed to explain the induction of tolerance in peripheral autoreactive T cells. The earliest model (2) proposes that the immune system is capable of responding only to nonself and not to self-Ags and that Ag-reactive cells themselves discriminate between self and nonself and make the decision whether or not to respond (3). Nevertheless, much evidence accumulated in the last 20 years (4) has led to the different assumption that the decision to initiate an adaptive immune response is not made by the Ag-specific cells but by the APCs (5–7). The concept is that the activation of a T cell response requires more than the Ag signal (signal one), and the outcome of the encounter between a T cell and an APC depends on the state of activation of the APC (signal two). In particular, the Danger Model emphasizes the problem of peripheral tolerance: in the presence of danger signals APCs are activated, express signal one and signal two, and are capable of activating T cells, whereas in the absence of danger APCs are not activated and (Ag-experienced or naive) T cells that interact with resting APCs die for lack of costimulation (8).

Therefore, two possible inductive processes of the tolerization phenomenon can be envisaged: 1) persisting Ags (self) can tolerate specific T cells independent of the activation state of APCs or 2) it is exclusively the state of differentiation of APCs that matters in the activation or inhibition of the adaptive immune responses. These two possible aspects of the tolerization process were never evaluated simultaneously and it has never been formally shown that persisting Ag per se can induce specific T cell tolerance independent of the state of activation of APCs. Effectively, when autoreactive T cells are challenged with a rapid increase of a persisting Ag, such as new autoreactive thymic emigrants that encounter the Ag in the periphery, the result of this interaction is an initial activation followed by tolerization (11–13). This phenomenon has been attributed both to the fact that the Ag is presented in the absence of signal two (inflammation) (14) or to Ag persistence (15). Moreover, the observation that viral or bacterial infections can interfere with the process of peripheral tolerance induction (13) would support the hypothesis that it is the Ag presentation in absence of inflammation that can induce tolerance. Nevertheless, it has been shown that in the case of persistent and overwhelming noncytopathic lymphocytic choriomeningitis virus...
infections, both virus-specific CD8+ and CD4+ T cells are rendered unresponsive (16). This phenomenon has been attributed to the persistence of the Ag, but still the possibility exists that non-professional or resting APCs present viral Ags and tolerate previously activated T cells (17). In this study we investigated whether chronic Ag presentation per se could be an inductive mechanism of peripheral T cell tolerization independent of the state of APC activation in conditions in which the only APCs are B cells. We set up an experimental model in which naive TCR transgenic (Tg)3 CD4+ T cells specific for the 435–451 peptide (Bpep) in the CH3 region of the IgG2a+ (18, 19) were transiently or chronically exposed to activated or resting Bpep-presenting B cells. We show that the persistence of Ag presentation could represent a major mechanism responsible for peripheral T cell tolerization independent of the activation state of Ag-presenting B cells.

Materials and Methods

Mice
BALB/c, C57BL/6, and SCID mice were purchased from Harlan Italy. RAG2+/− BALB/c and C57/129-Tg mice were obtained from The Jackson Laboratory. MHC class II and β2-microglobulin double knockout (KO) (βII+) mice were from Centre de Distribution, de Typage et d’Archivage d’Animaux (Orleans, France). TCR α-chain-deficient (αKO) BALB/c mice (αKO-b−) were provided by Dr. M. J. Owen (Imperial College, London, U.K.). They were backcrossed on CB-17 background to obtain αKO-b− mice. αKO-b− mice were bred with αKO-b− to obtain αKO-CB-17 × αKO-BALB/c F1 mice. αKO-b− and αKO−b+ were mated with anti-IgG2a+ Tg mice to obtain αKO-Tg+ b− and αKO-Tg+ b− mice. I-β2F−GFP mice were provided by Dr H. Ploegh (Harvard Medical School, Boston, MA). All animals were kept in specific pathogen-free conditions.

Cell preparations

Naïve TCR Tg anti-IgG2a+ T cells (2a T cells) were purified from spleen and lymph nodes of αKO-Tg+ b− mice. A total of 106 cells/ml were stained with biotinylated anti-CD8, anti-CD11c, anti-CD11b, and anti-GR1 Abs (20 μg/ml), washed and then incubated with streptavidin MicroBeads (Miltenyi Biotec). Cells were then negatively selected on MS MACS separation columns according to Miltenyi Biotec instructions.

Small resting B cells were isolated from spleens of BALB/c mice. Splenic unicellular cell suspensions were incubated with anti-B220 (20 μg/ml) and anti-CD11c (20 μg/ml) Abs, and cells were sorted by gating the small B220+ and CD11c+ populations (purity 99%).

LPS-activated B cells were obtained from spleen of BALB/c or CB-17 mice. Splenic unicellular suspensions were plated at a density of 1 × 106 cells/ml in IMDM (Sigma-Aldrich) supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Sigma-Aldrich), and 10% heat-inactivated FBS (complete IMDM) (1 × 106 cells/ml) in the presence of LPS (10 μg/ml) and IFN-γ. On the day of injection, V14 down-regulation on 2a T cells was analyzed by FACS.

2a T cells were purified by magnetic negative selection from lymph nodes of CB-17 mice and loaded with LPS-activated B cells (1 × 106 cells/mouse) and treated with IFN-γ, anti-CD44. FACS analysis was performed using CD44, anti-mouse CD44. FACS analysis was performed using BD Biosciences FACScan and CellQuest software. Cell sorting was performed using a MoFlo FACS.

In vitro T cell responsiveness

2a T cells were purified, as earlier described, from transferred mice 1 mo after transfer. Purified 2a T cells (1 × 106 cells/mouse) were reconstituted in complete IMDM, plated in 24-well plates, and Bpep was added at different concentrations. After 4 h of coculture, Vβ14 down-regulation on 2a T cells was analyzed by FACScan. After 24 h, clarified supernatants were tested for IFN-γ production, using IFN-γ Duo ELISA kit (R&D Systems).

Anti-KLH IgG2a

CB-17 mice were immunized s.c. with 200 μg of KLH (Sigma-Aldrich) in CFA (Sigma-Aldrich). Two weeks later, B and T cells from draining lymph nodes were purified as described, and injected (4 × 106 cells/mouse) i.p. in SCID mice together with 2a T cells (1.5 × 106 cells/mouse). Transferred SCID mice were then i.p. injected with 200 μg of KLH in PBS, and 10 days later serum levels of anti-KLH IgG2a+ were measured by ELISA as previously described.

APC activation state experiments

The 2a T cells were purified by magnetic negative selection from lymph nodes and spleen of αKO-Tg+ b− mice and injected i.v. (105 in 200 μl of PBS/mouse) into αKO-CB-17 × αKO-BALB/c F1 mice. Recipient animals were then subdivided in five subgroups and injected, respectively, with: small resting B cells (105 in 100 μl of PBS/mouse) purified by sorting, as previously described, from spleens of CB-17 mice and loaded with Bpep; small resting B cells not-pulsed with Bpep (as a control, 105 in 100 μl of PBS/mouse); LPS-activated B cells (105 in 100 μl of PBS/mouse) derived from CB-17 mice and loaded with Bpep; LPS-activated B cells (105 in 100 μl of PBS/mouse) derived from BALB/c mice and treated with IFN-γ; and PBS only. Recipient animals received four injections of the indicated population of B cells; the first one was combined with the 2a T cells adoptive transfer the other three were performed at 2-day intervals.

One month after transfer, blood samples were taken from mice to execute a cytometric analysis and to measure the IgG2a+ serum levels. Later, all the animals were sacrificed and 2a T cells purified and injected into SCID mice to test their capacity to inhibit anti-KLH IgG2a+ -producing B cells in vivo as indicated. The same procedure was used when naïve 2a T cells were injected into βII+ mice with the difference being the cells being the cells injected once or repetitively.

Results

The activation state of APCs is not relevant for the tolerization process

To investigate whether the activation state of APCs could influence the peripheral T cell tolerization when the Ag is chronically

Abbreviations used in this paper: Tg, transgenic; Bpep, 435–451 peptide of IgG2a; KO, knockout; KLH, keyhole limpet hemocyanin; DC, dendritic cell.
presented, an experiment was performed in haplotype-matched mice. We have shown in our companion study that chronic Ag presentation by B cells is required to induce peripheral Ag-specific T cell tolerance. In particular, we performed experiments in which naive CD4\(^{+}\) T cells (2a T cells) specific for the 435–451 peptide (Bpep) of IgG2ab were transferred to mice showing persistent or transient Bpep presentation by B cells. 2a T cells are able to kill Bpep-presenting B cells (and we have previously shown (18) that only IgG2a\(^{+}\) B cells are able to present the Bpep) (20). When Ag-presenting B cells are found in low frequency they quickly disappear after 2a T cell encounter, and this transient B cell Ag presentation leads to T cell activation and eventually to a T memory phenotype. In contrast, when Bpep-presenting B cells are highly frequent they can persist, and Ag-specific T cells are tolerized. In the present work, we investigate whether the activation state of Ag-presenting B cells could influence the peripheral T cell tolerization process. To this purpose, we transferred naive 2a T cells from TCR Tg \(\alpha\)KO-BALB/c mice (expressing the \(\alpha\) allotype of the IgG2a that is not recognized by the Tg TCR) into TCR \(\alpha\)-chain-deficient (\(\alpha\)KO) mice expressing the b and a allotype of the IgG2a Ig and thus able to present the Bpep (\(\alpha\)KO-CB-17 \(\times\) \(\alpha\)KO-BALB/c \(\alpha\)F, mice). We have shown in our companion study that the 2a T cells transferred into the \(\alpha\)KO-CB-17 \(\times\) \(\alpha\)KO-BALB/c \(\alpha\)F mice, in the absence of inflammation at steady state conditions, are not tolerized because of the transient presentation of Ag. We also have shown that 2a T cells can be tolerized in these \(\alpha\)F recipients if the persistence of Ag-presenting B cells is increased by repetitive injections of Bpep-presenting small resting B cells. To investigate whether the B cell activation state could influence the T cell tolerization process, \(\alpha\)KO-CB-17 \(\times\) \(\alpha\)KO-BALB/c \(\alpha\)F mice that received 2a T cells were subjected to injections of activated or small resting Bpep-presenting B cells every other day for a total of four injections. LPS-activated B cells used had high levels of MHC and costimulatory B7-1 and B7-2 molecules, whereas small resting B cells expressed very low amounts of these molecules at the cell surface (Fig. 1). In particular, two groups of recipient animals received LPS-activated B cells, either of b haplotype (presenting the Bpep) or control a haplotype, which were switched in vitro to IgG2a in the presence of IFN-\(\gamma\). Two other groups received small resting B cells either pulsed or not pulsed with the Bpep (Fig. 2A). One month after transfer, a clear population of expanded 2a T cells, corresponding to ~40% of total CD4\(^{+}\) cells, was present in the lymph nodes of all of the groups of recipient mice (data not shown) and showed high CD44 levels (Fig. 2B). These cells clearly underwent Ag-driven proliferation because when 2a T cells were transferred into \(\alpha\)KO BALB/c mice not expressing the cognate Ag, very few of them (~1% of total CD4\(^{+}\) cells) could be observed in the lymph nodes (data not shown).
In vitro analysis of transferred T cell responsiveness

Functional activity of the transferred 2α T cells was then tested in vitro. One month after transfer into αKO-CB-17 × αKO-BALB/c F1 mice, 2α T cells were recovered from lymph nodes of the four groups of transferred mice, rechallenged with Bpep-loaded B cells in vitro, and their responsiveness measured by means of TCR down-regulation and IFN-γ production. We took these two parameters into account as an index of 2α T cell activation because we observed that functional 2α T cells strongly down-regulated their TCR in vivo with a very low amount of peptide in vitro. Other parameters, such as CD69 and CD25 up-regulation, could not be taken into account because the TCR down-regulation hampered a proper identification of the cells. The functional activity of recovered 2α T cells in vitro was compared with naive and memory 2α T cells because it is well established that memory cells can be activated in vitro at lower Ag dose compared with naive T cells (21, 22). The 2α T cells recovered from hosts that received injections of control small resting B cells or control B cell blasts behaved in vitro like memory cells. In contrast, 2α T cells derived from hosts that received repetitive injections of Bpep-presenting small resting B cells did not show any activity (Fig. 3). Surprisingly, even treatment with Bpep-presenting B cell blasts induced the generation of a population of nonresponding 2α T cells (Fig. 3). Thus, chronic Bpep presentation rendered 2α T cells nonresponsive even in the presence of B cells that received inflammatory signals and were properly activated.

In vivo analysis of transferred T cell functionality

Anti-IgG2abα T cells show a killing activity vs IgG2ab+ B cells (20). Therefore, we investigated in vivo the functionality of 2α T cells, 1 mo after transfer, by analyzing their ability to suppress IgG2ab+ B cells. A preliminary indication was derived from the measurement of IgG2ab serum levels in the different recipients. As shown in Fig. 4A, both mouse groups treated with Bpep-presenting small resting B cells or Bpep-presenting LPS-activated B cells had measurable levels of IgG2ab in the serum. In contrast, IgG2ab was completely suppressed in mice treated with control small resting or activated B cells (Fig. 4A), suggesting that 2α T cells were tolerized both in mice treated with Bpep-presenting activated B cells and in mice treated with Bpep-presenting resting B cells. To confirm this prediction, we tested the capacity of transferred 2α T cells to inhibit Ag-specific IgG2ab+ B cells in vivo. Thus, 1 mo after transfer, 2α T cells from the four groups of animals (Fig. 4B) were transferred to SCID mice together with KLH-sensitized T and B cells from CB-17 mice (potentially able to produce anti-KLH IgG2ab responses), and recipient animals were challenged with KLH. Ten days later anti-KLH IgG2ab serum levels were measured (Fig. 4B). As suggested by the previous results, SCID mice that received 2α T cells from animals treated with Bpep-presenting activated B cells or Bpep-presenting resting B cells were both able to mount an anti-KLH IgG2ab response, whereas in SCID mice that received T cells from control groups the anti-KLH IgG2ab response was suppressed (Fig. 4C). Altogether these results indicated that 2α T cells were tolerized in conditions of chronic Ag presentation independent of the state of activation of Ag-presenting B cells.

Tolerized 2α T cells did not acquire a regulatory activity as shown by their inability to produce IL-10 and to suppress the functionality of naive 2α T cells in vitro and in vivo (data not shown).

The activation state of Ag-presenting B cells is not relevant for the tolerization process when the Ag is chronically presented

To better investigate whether the activation state of Ag-presenting B cells was not relevant for T cell tolerization when the Ag is chronically presented, an experiment was performed in βII+ mice. Although we have never observed presentation of Bpep by non-IgG2ab+ APCs in H-2d background (18), we could not formally exclude that in any of the conditions tested endogenous resting APCs could present the Bpep and be responsible for the tolerization process. To formally exclude this point, we repeated the previous experiment using βII+ mice as recipients, whose endogenous APCs were consequently unable to cross-present the Bpep. Moreover, we have previously shown that B cells not producing the IgG2ab are not able to present this Ig, neither in vitro nor in vivo because they do not express Ig receptors and are not able to internalize exogenous Igs (18). For this reason, in this particular experimental setting, the IgG2ab molecule eventually secreted by the injected IgG2ab+ producers could not be presented by other coinjected IgG2ab-negative B cells but only by IgG2ab-positive B cells.

![FIGURE 3](http://www.jimmunol.org/) In vitro analysis of 2α T cell functionality after repetitive encounter of small resting B cells (RB) or activated B cell (Blasts) presenting the Bpep. The ability of 2α T cells, recovered 1 mo after transfer in αKO-CB-17 × αKO-BALB/c F1 animals, to respond in vitro to APCs in presence of graded amounts of Bpep. Naive and memory cells were added as controls. TCR down-regulation (left panel) measured 4 h after coculture with APCs in presence of Bpep, the percentage of 2α T cells showing high levels of Vβ8.14 expression was investigated by FACS analysis. IFN-γ levels in coculture supernatants (right panel) were measured by ELISA at 24 h. Data represent 2α T cells recovered after transfer in mice that received repetitive injections of small resting B cells presenting the Bpep (RB Bpep); 2α T cells recovered after transfer in mice that received repetitive injections of small resting B cells not presenting the Bpep (RB Ctl); 2α T cells recovered after transfer in mice that received repetitive injections of IgG2a-switched and activated B cells from CB-17 mice (Blasts IgG2a); and 2α T cells recovered after transfer in mice that received repetitive injections of IgG2a-switched and activated B cells from BALB/c mice (Blasts Ctl).
To first check in vivo the persistence and activation state of B cells transferred into \( \beta'^{-}\)II' mice, we did single and repetitive injections of small resting B cells or LPS blasts expressing the GFP under the MHC class II promoter control. GFP expression allowed us to easily track them in vivo. We observed that the injected B cells, both naive and activated, persisted for a maximum of 2 days. Naive cells maintained their resting phenotype (Fig. 5A). This experimental setting permitted a good control of both the state of activation and the persistence of B cells that correlated with the number of injections. In this way, we were able to directly link the persistence of Ag presentation and the state of B cell activation with the fate of anti-IgG2a \(^b\) T cells.

\( \beta'^{-}\)II' mice were transferred with naive 2a T cells and divided in five groups. One group received a single injection of small resting B cells loaded with the Bpep, while a second group received a single injection of IgG2ab-switched LPS B cell blasts. The third and the fourth groups received a total of four injections, one every other day, of Bpep-loaded small resting B cells or IgG2a\(^b\)-switched...
B cell blasts, respectively. The last group did not receive any B cells (Fig. 5B). One month later, we tested the recipients for the presence of transferred T cells. Similar to the results of the experiment shown in Fig. 2B, T cells were present in the lymph nodes of the first four groups of mice (data not shown) and they were all CD44high (Fig. 5C). We could exclude that these cells underwent homeostatic proliferation (23) because the mice that did not receive B cells did not show any T cells in lymph nodes (data not shown).

**In vitro analysis of transferred T cell responsiveness**

The functionality of naive T cells 1 mo after transfer in βII* mice was then tested in vitro as described in the previous experiments. The naive T cells were recovered from the lymph nodes, challenged with Bpep-loaded B cells, and their responsiveness measured by investigating TCR down-regulation. As shown in Fig. 6A, naive T cells derived from hosts that received a single injection of Bpep-presenting B cells were able to respond to in vitro restimulation. In contrast, naive T cells derived from hosts that received repetitive injections of Bpep-presenting B cells (small resting or blasts) did not show any activity. This result indicates that chronic Bpep presentation either by resting or activated B cells rendered, by itself, naive T cells nonresponsive.

**In vivo analysis of transferred naive T cell functionality**

In vivo 2a T cell functionality was then tested by analyzing their ability to suppress the IgG2a anti-KLH response (Fig. 6B). As shown in Fig. 6C, naive T cells were able to suppress the IgG2a B cell activity if they were recovered from mice treated with a single injection of either small resting B cells or activated LPS B cell blasts. Conversely, naive T cells were nonfunctional if they were derived from mice that received repetitive injections. Thus, the activation state of Ag-presenting B cells was not relevant for the peripheral T cell tolerization process in the context of chronic Ag presentation.

**Discussion**

The goal of this study was to investigate whether the persistence of Ag presentation per se could be an inductive mechanism of the T cell tolerization process even when the Ag is presented by activated APCs. To this aim, we transferred naive T cells specific for an epitope in the CH3 region of IgG2a in mice in which these cells could be transiently or chronically exposed to the Ag presented by resting or activated B cells. As already observed in a previous study (24), T and B cells find each other efficiently in lymph nodes and in the spleen. In our experimental system, we have observed that when B cells are the exclusive APCs and the Ag is persistently presented either by small resting B cells, showing low levels of MHC class II and costimulatory molecules, or by activated B cells, having high levels of MHC and costimulatory molecules, the autoantigen-reactive T cells become unresponsive. The intervention of non-B resting APCs was definitively excluded in adoptive transfer experiments performed in βII* mice in which...
cross-presentation of IgG2a b peptides by endogenous resting APCs was not permitted. These results suggest that tolerance to peripheral self-Ags can be achieved in the presence of activated Ag-presenting B cells provided that the Ag is continuously presented for a sufficient period of time. This implies that autoreactive new thymic emigrants that encounter the Ag in the periphery can be tolerized by chronic Ag exposure. In our experimental system, B cells were polyclonally activated using LPS. Thus, we cannot exclude the possibility that B cells would not be able to induce T cell tolerance if they are activated via the BCR.

In a previous work, we have shown that the functionality of 2a T cells matured in CB-17 mice (presenting the cognate Ag) differed depending on whether they were kept in sterile or dirty housing conditions (25). Although no T cells responded to peptide under sterile conditions, some T cell responsiveness was observable after 3 mo of dirty housing or immunization and led to in vitro proliferation and suppression of serum IgG2ab. This observation could possibly contrast with the present results. Nevertheless, in that particular work, we could not distinguish whether inflammation recovered tolerant T cells or whether it interfered with the tolerization process because we could not exclude the continuous intervention of new thymic emigrants. In this study, we have performed adoptive transfer experiments to follow the fate of naïve T cells in absence of new thymic emigrants and unrecoverable T cell tolerance occurs.

It would be of interest to investigate whether the phenomenon that we describe (independence of the state of APC activation to induce T cell tolerance when the Ag is chronically presented) could be extended also to cases in which the Ag is presented by non-B APCs, such as dendritic cells (DCs). Recent data, concerning the ability of DCs to induce peripheral T cell tolerance, originate from experiments in which only immature DCs can induce T cell unresponsiveness (14). In particular, immature or CD40-activated DCs are targeted with the Ag in vivo, and the fate of CD4+ and CD8+ Ag-specific T cells followed over time (14, 26). As T lymphocyte tolerization is observed only when immature Ag-loaded DCs are encountered, the immature state of these APCs, characterized by the absence of sufficient costimulation and sufficient signal two, is thought to be responsible for this process. A second evidence for the capacity of immature DCs to induce peripheral T cell tolerance depends on their ability to cross-present peripheral tissue Ags and induce abortive T cell activation (27). In both these cases, DC Ag presentation is constitutive and persistent (28). Thus, as in the previous experiments, one possible interpretation is that T cell tolerance is due to Ag presentation by nonactivated DCs that do not exhibit sufficient signal two. Nonetheless,
although the activation state of DCs has been described to be extremely relevant for the decision to suppress or activate an immune response, the hypothesis that chronic Ag presentation per se could induce tolerance has not been clearly investigated.

To properly clarify this point, we are currently performing experiments in which presentation of self-Ags by DCs in the presence or absence of activation stimuli is transient or persistent in inducible Ag-Tg mouse models.

There is evidence that T cell interactions with immature or semimature DCs may induce peripheral differentiation/expansion of regulatory T lymphocytes, an alternative mechanism to maintain peripheral tolerance (29, 30). In our experimental model, we have never observed the differentiation of regulatory T cells in vivo. In particular, no IL-10-producing lymphocytes have been detected in the population of nonfunctional T cells after transfer in high Ag concentration and that are only presented for a short period of time.

The hypothesis that chronic Ag presentation per se could induce tolerance has not been clearly investigated.

To properly clarify this point, we are currently performing experiments in which presentation of self-Ags by DCs in the presence or absence of activation stimuli is transient or persistent in inducible Ag-Tg mouse models.

In conclusion, our study suggests that when the main APCs are B cells, the major mechanism responsible for peripheral T cell tolerization is the persistent Ag exposure, independent of the state of activation of Ag-presenting B cells. This would imply that for the adaptive immune system self-Ags are Ags that are chronically presented, such as Ags that slowly accumulate or that persist after a rapid increase, whereas nonself Ags are Ags that rapidly change in concentration and that are only presented for a short period of time.

Disclosures
The authors have no financial conflict of interest.

References