Follicular DCs (FDCs) play a central yet incompletely understood role in the adaptive immune response (for review see Allen and Cyster, 2008). As a major stromal cell constituent of both primary and secondary follicles, FDCs secrete chemokines such as CXCL13 to spatially organize the intrafollicular migration of B cells and follicular helper T cells. In addition, FDCs have the unique capacity to retain immune complexes on their cell surface for week- to month-long periods by virtue of their expression of FcγRIIb Fc receptors and CR1 (CD35) and CR2 (CD21) complement receptors. These immune complexes play a key role during the germinal center reaction, as they provide the antigenic substrate that drives antibody affinity maturation. Conversely, follicular B cells produce lymphotoxin α1β2 (LTα1β2), which acts as a key FDC survival factor.

Follicular DCs (FDCs) are prominent stromal cell constituents of B cell follicles with the remarkable ability to retain complement-fixed antigens on their cell surface for extended periods of time. These retained immune complexes have long been known to provide the antigenic stimulus that drives antibody affinity maturation, but their role in cellular immunity has remained unclear. In this study, we show that FDC-retained antigens are continually sampled by lymph node–resident DCs for presentation to CD8 T cells. This novel pathway of antigen acquisition was detectable when FDCs were loaded with purified antigens bound into classical antigen–antibody immune complexes, as well as after pregnancy, when they are loaded physiologically with antigens associated with the complement-fixed microparticles released from the placenta into maternal blood. In both cases, ensuing antigen presentation was profoundly tolerogenic, as it induced T cell deletion even under inflammatory conditions. These results significantly broaden the scope of FDC function and suggest new ways that the complement system and persistent antigen presentation might influence T cell activation and the maintenance of peripheral immune tolerance.

Contributions of FDCs toward T cell immunity have been much less apparent. FDCs do not express MHCII molecules nor do they have the capacity to phagocytose and process exogenous antigens for MHCI-restricted presentation (Schnizlein et al., 1985; Gray et al., 1991). Thus, it is unlikely that these cells present exogenous antigens to T cells. However, FDC-bound antigen has been indirectly implicated in the extended phases of antigen presentation considered important for optimizing CD4 T cell memory (van Essen et al., 2000). Moreover, it has recently been shown that FDC-retained antigens, in the form of antigen–antibody immune complexes, can be acquired by cognate B cells for processing and presentation to follicular helper T cells (Suzuki et al., 2009).
These results extend older experiments demonstrating that the ∼0.3-µm-diameter immune complex–bearing bodies known as iccosomes can be acquired from the FDC cell surface by both germinal center B cells and tingible body macrophages (Szakal et al., 1988). However, the possibility that DCs might also acquire FDC-bound immune complexes has not been directly addressed. Such an antigen transfer pathway would give FDC-bound antigens general access to both MHC1 and MHCII presentation pathways and to a cell type with a central role in regulating both CD8 and CD4 T cell responses.

In the course of our previous work on the pathways that mediate the presentation of fetal/placental antigens during pregnancy, we noticed that the transgenic expression of a transmembrane form of the model antigen OVA by cells of the mouse conceptus not only induced the systemic proliferation of antigen-specific maternal T cells but also led to the accumulation of OVA+ immunoreactive material on FDCs throughout all maternal secondary lymphoid organs (Erlebacher et al., 2007). These experiments involved mating wild-type females to males bearing the Act-mOVA transgene, which directs relatively ubiquitous OVA expression from the β-actin promoter (Ehst et al., 2003). Most likely, OVA had access to all maternal secondary lymphoid organs because the cell types expressing the transgene included labyrinthine trophoblasts, which during development establish a massive degree of surface contact with maternal blood, as well as endovascular placental trophoblasts directly at the maternal/fetal interface, which showed particularly high transgene expression levels (Erlebacher et al., 2007). Moreover, it was likely that OVA was being shed into maternal blood through a process analogous to the hematogenous release of subcellular membranous material from the syncytiotrophoblast layer of the human placenta (Redman and Sargent, 2007). This release generates a substantial amount of placenta-derived microparticles in the blood of pregnant women (Taylor et al., 2006).

The binding of shed placental material to FDCs thus presented a physiological context for evaluating how FDC-bound antigen might influence T cell behavior. As might be expected, this material remained associated with FDCs for at least several weeks after delivery. Surprisingly, however, the presentation of shed placental OVA to CD8 T cells likewise persisted for several weeks into the postpartum period, far longer than the lifespan of any individual secondary lymphoid organ–resident DC that might have ingested antigen during gestation. Further experiments revealed that this extended phase of antigen presentation was caused by the continual sampling of FDC-associated OVA deposits by progressive cohorts of bone marrow–derived DCs. This novel pathway of antigen acquisition by DCs could also be detected in virgin mice bearing FDCs loaded with classical OVA–anti-OVA antigen–antibody immune complexes. In both cases, ensuing antigen presentation was highly tolerogenic as it led to the deletion of naive OVA-specific CD8 T cells even in the presence of adjuvant-induced inflammation.

RESULTS
A prolonged period of postpartum antigen presentation correlates with the retention of shed placental antigen on LN FDCs
In accord with our previous results (Erlebacher et al., 2007), mating wild-type females to Act-mOVA transgenic males but not to control C57BL/6 (B6)–mated females led to the appearance by late gestation of OVA+ immunoreactive material on CD35+ FDC networks throughout all maternal secondary lymphoid organs, including the subcutaneous LNs and spleen (Fig. 1 A, top and middle). These OVA+ FDC networks were still visible 3 wk after delivery (Fig. 1 A, bottom, arrows), and their existence surprisingly correlated to extended phases of OVA presentation to maternal T cells. Thus, naive CD8+ OT-I TCR transgenic T cells (Hogquist et al., 1994), which recognize the OVA257–264 peptide presented by H-2Kb (and which were crossed onto a B6CBF1 background for transfer into the B6CBF1 females used in these experiments), underwent antigen–driven proliferation when the cells were adoptively transferred into Act-mOVA–mated females 3–4 wk after delivery (Fig. 1 B; representative CFSE dilution plots are shown in Fig. 1 C). Robust OT-I proliferation (defined here as being the case when >25% of the cells divided more than once) and clear OVA+ FDC networks were frequently seen in the subcutaneous LNs of postpartum Act-mOVA–mated females, whereas persistent OT-I proliferation and OVA+ FDC networks were much less apparent in the spleen. OVA+ FDC foci in the spleen, when detectable, were also much smaller than in the LNs.

The aforementioned data revealed an anatomical correlation, and thus a potential link, between antigen retention on FDCs and persistent antigen presentation. This potential link was further strengthened by several additional considerations. First, we found that the intravenous injection of virgin or postpartum B6–mated females with 100 µg soluble OVA only induced an acute OT-I proliferative response that declined virtually to baseline levels by 7 d (Fig. S1 A). This result is consistent with the estimated 2–14-d lifespan of lymphoid organ–resident DCs (Kamath et al., 2002; Liu et al., 2007), which are the cells most often implicated in cross-presenting blood-borne antigens to naive CD8 T cells, and indicated that the postpartum period was not associated with a general extension of APC lifespan. Second, serum OVA levels in late gestation Act-mOVA–mated females were below our limit of detection by ELISA (10 ng/ml; unpublished data), and both pure OVA protein and soluble OVA–containing immune complexes are known to be rapidly cleared from the blood (t1/2 < 1 h) when injected intravenously at doses below ∼1 mg/mouse (Kim and Halsey, 1982; Carter et al., 1984). Third, persistent OVA presentation could not be explained by the protracted release of residual OVA from the postpartum uterus, as Act-mOVA–mated females subjected to hysterectomies within 1 wk of delivery still showed robust OT-I proliferation in the subcutaneous LN and OVA immunoreactivity on LN FDCs when assessed 3 wk later (Fig. S1, B and C). Indeed, the observation of persistent OVA presentation in the LN but not the spleen was highly inconsistent with OVA being continuously released into the blood.
from a peripheral tissue reservoir or persisting at low levels within the blood itself. Instead, these data together suggested that the extended phase of OVA presentation in the postpartum period was caused by APCs persistently sampling depots of shed placental OVA retained within the LN themselves and raised the possibility that these depots were FDC associated.

**Persistent presentation of placental OVA is mediated by bone marrow–derived APCs that ingest antigen de novo in the postpartum period from a radioreistant depot**

To substantiate the idea that the APCs presenting OVA ≥3 wk after delivery had in fact acquired antigen in the postpartum period, we assessed postpartum OT-I proliferative responses in Act-mOVA–mated females under conditions in which the APCs expressing the H-2Kb MHCI restriction element required for OVA presentation to OT-I cells would themselves only exist in the postpartum period. To this end, we first generated H-2Kb-deficient mice on a B6CBAF1 background by mating B6 background MHCI-deficient H-2Kb−/− H-2Db−/− (Kb−/− Db−/−) mice (Pérarnau et al., 1999) with CBA/J mice, which bear a H-2k haplotype. The resultant Kb−/− Db−/− B6CBAF1 mice, which as expected showed no antigen-specific OT-I proliferative response after soluble OVA injection (Fig. 2A), were mated to Act-mOVA or control B6 males and then

**Figure 1. OVA expression by the conceptus induces OVA accumulation and retention on FDCs and an extended phase of OVA presentation in the postpartum period.** (A) B6CBAF1 females were mated to either B6 or Act-mOVA males, and secondary lymphoid organs were collected either in late gestation (E17.5–18.5; top two rows) or 3 wk after delivery (bottom row). Serial tissue sections were stained with anti-CD35 antibodies (green) to identify FDC networks and anti-OVA antibodies to identify OVA deposits (red) and were counterstained with DAPI (blue). Arrows indicate OVA+ immunoreactive material present at 3 wk postpartum. During gestation, OVA+ FDC networks were occasionally also visible in Act-mOVA–mated mice (arrowhead). Data are representative of at least n = 4 mice per group and at least three independent experiments. (B and C) OVA presentation in the subcutaneous LNs (scLN) and spleens of 3–4 wk postpartum females. Antigen presentation was assessed by injecting the mice with CFSE-labeled OT-I T cells and then visualizing cell proliferation 44 h later by flow cytometry. B shows, for individual mice, the percentage of OT-I cells having undergone more than one division cycle (n = 5–7 mice per group compiled over two independent experiments), whereas C shows representative CFSE dilution plots.
given 4–5 × 10⁶ wild-type B6CBAF1 bone marrow cells within 1 wk after delivery to generate H-2Kb-expressing APCs. OT-I T cells were then transferred 2 wk after bone marrow transfer (i.e., 15–20 d after delivery), and their proliferative response in the subcutaneous LNs was assessed 44 h later. Because FDC-associated antigen depots can be obliterated by high doses of irradiation (Phipps et al., 1981), the conditioning regimen used in these experiments used a combination of cyclophosphamide administration, NK cell depletion, and sublethal irradiation at a dose (600 cGy) that was empirically established to give a reasonable rate of transplant acceptance while simultaneously preserving OVA+ FDC networks (Fig. S2).

Not surprisingly, OT-I proliferation was undetectable in the n = 2 postpartum Act-mOVA–mated Kb–Dkb– B6CBAF1 females in which the wild-type bone marrow transfer gave only low levels (i.e., 2 and 30%) of donor cell contribution to the total CD11c+ MHCII+ DC pool (as assessed in spleen cell preparations; unpublished data). Strikingly, however, Kb–Dkb– B6CBAF1 females with high levels of DC chimerism (i.e., with 77–99% of DCs being of wild-type, donor origin; Fig. S2) showed robust OT-I proliferation 3 wk after delivery in 5 out of n = 6 mice (Fig. 2 B; representative CFSE dilution plots are shown in Fig. 2 C). T cell proliferation was antigen driven, as it was not evident in B6 control–mated females (n = 5), although many of the OT-I cells in both sets of mice underwent one round of division, which is consistent with their lymphopenia-induced homeostatic expansion. Taken with our previous evidence that fetal cells do not directly present fetal/placental OVA to maternal T cells (Erlebacher et al., 2007), these data formally demonstrated the potential for bone marrow–derived APCs generated in the postpartum period to acquire antigen from a long-lived antigen depot generated during pregnancy. Conversely, the preservation of this OVA depot after a radiation–based bone marrow–conditioning regimen implied that the depot was associated with a radiosensitive cell type such as an FDC rather than a bone marrow–derived cell type.

**Persistent presentation of placental antigen in the postpartum period is abrogated by the administration of a lymphotixin β receptor (LTβR)–Ig fusion protein**

To further implicate FDC-bound OVA as the antigen source for persistent presentation, we assessed OT-I proliferative responses in postpartum Act-mOVA–mated females treated with LTβR–Ig. This reagent acts as a decoy receptor for the FDC survival factor lymphotixin LTα1β2 and thus induces the rapid regression of FDC networks and the dissipation of associated immune complexes (Mackay and Browning, 1998). Accordingly, Act-mOVA–mated females given a single injection of LTβR–Ig within 1 wk after delivery showed no OVA deposits in the subcutaneous LN 3 wk later (i.e., 21–26 d after delivery), although CD35+ FDC networks had largely recovered by this point (Fig. S3). Strikingly, LTβR–Ig treatment also induced a complete loss of postpartum OT-I proliferative responses (Fig. 3 A; representative CFSE dilution plots are shown in Fig. 3 B). In contrast, OVA+ FDC networks as well as OT-I proliferative responses were still apparent in Act-mOVA–mated mice injected with control polyclonal human IgG (hIgG). Disruptions in LTα1β2-induced signaling are known to affect DC homeostasis as well as cells such as subcapsular sinus macrophages that help mediate the initial transport of immune complexes to FDCs (Kabashima et al., 2005; Suzuki et al., 2009). However, postpartum B6–mated mice showed no detectable impairment in the presentation of soluble OVA injected 3 wk after LTβR–Ig treatment.

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**Figure 2.** Placental OVA is retained by a radiosensitive cell type and can be acquired by bone marrow–derived APCs generated after parturition. (A) Absence of OVA presentation to OT-I cells in Kb–Dkb– B6CBAF1 mice. Virgin Kb–Dkb– B6CBAF1 and B6CBAF1 mice were either left untreated or intravenously injected with 100 μg OVA. After 4 h, the mice were given CFSE-labeled OT-I cells. T cell proliferation was then visualized in the subcutaneous LNs 44 h later by flow cytometry. Data are from two independent experiments and are representative of n = 2 mice in both the untreated groups, and n = 3 and n = 5 mice in the OVA-injected B6CBAF1 and Kb–Dkb– B6CBAF1 groups, respectively. (B and C) OVA presentation in Act-mOVA–mated Kb–Dkb– B6CBAF1 females subjected within 1 wk after delivery to a radiation-based bone marrow-conditioning regimen and reconstitution with wild-type B6CBAF1 bone marrow. 2 wk after transplantation (i.e., 15–20 d after delivery), antigen presentation was assessed in the subcutaneous LNs by injecting the mice with CFSE-labeled OT-I T cells and then visualizing cell proliferation 44 h later by flow cytometry. B6–mated Kb–Dkb– B6CBAF1 females were subjected to the same protocol and served as controls. B shows, for individual mice, the percentage of OT-I cells having undergone more than one division cycle (n = 5–6 mice per group compiled over three independent experiments), whereas C shows representative CFSE dilution plots.
that both C3 and C4 activation products can directly bind CR1/2 (Selander et al., 2006). Indeed, mice with a targeted deletion of the Cr2 gene (Ahearn et al., 1996), which encodes both CR1 and CR2 receptors in mice, showed a dramatic, 96% reduction in the number of OVA+ FDC foci per splenic cross section (Fig. 4 A).

Importantly, none of these complement system deficiencies appreciably reduced the systemic proliferation of OT-I T cells adoptively transferred in late gestation, when antigen presentation was likely mediated predominantly by DCs that had directly acquired antigen from the blood (Fig. 4 B). Although the complement system was thus critical for efficient binding of shed placental material to FDCs, it was not involved in antigen shedding per se. Similarly, we observed systemic OT-I proliferation, and thus OVA shedding, in late gestation Act-mOVA–mated females variously deficient in B cells (µMT mice; Fig. 4 B), perforin (Pfp−/− mice), or NK cells (Il15−/− mice; Fig. S4).

OVA shed from the placenta binds maternal FDCs in a complement–dependent fashion

Given the critical role of complement in antigen binding by FDCs, we next assessed placental OVA shedding and the binding of shed OVA to FDCs in late gestation Act-mOVA–mated females deficient in various components of the complement system. As shown in Fig. 4 A, the number of OVA+ FDC foci visible per splenic cross section was reduced ~70% in C1qa−/− mice (Botto et al., 1998) deficient in the complement component C1q, which is a key initiator of the classical pathway, as well as in mice deficient in C4 (Wessels et al., 1995), which is a component of both classical and mannose-binding lectin pathways. The number of OVA+ FDC foci per splenic cross section was also reduced ~50% in mice deficient in C3 (Wessels et al., 1995), which is the central component of the classical, alternative, and mannose-binding lectin pathways, although the reduction did not reach a P < 0.05 level of statistical significance. Most likely, the moderate effect of each individual complement component deficiency reflected the existence of bypass complement pathways and the observation that both C3 and C4 activation products can directly bind CR1/2 (Selander et al., 2006). Indeed, mice with a targeted deletion of the Cr2 gene (Ahearn et al., 1996), which encodes both CR1 and CR2 receptors in mice, showed a dramatic, 96% reduction in the number of OVA+ FDC foci per splenic cross section (Fig. 4 A).

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Classical antigen–antibody immune complexes retained by FDCs are also persistently sampled by bone marrow–derived APCs

To determine whether the sampling of FDC-bound antigen by bone marrow–derived APCs was relevant only to placental-derived material and/or the postpartum period, we next assessed whether it was also apparent in virgin mice bearing classical antigen–antibody immune complexes. As shown in Fig. 5 A, B6CBAF1 virgin females showed clear OT-I proliferative responses in the subcutaneous LNs 3 wk after their intravenous injection with 100 µg soluble OVA and 250 µg rabbit anti-OVA antibodies. This extended phase of presentation was coincident with the long-term retention of

Figure 3. The extended phase of postpartum OVA presentation is abrogated in mice treated with LTβR-Ig. (A and B) Females were mated to either B6 or Act-mOVA males and within 1 wk after delivery were injected with either control hIgG or LTβR-Ig. 21 d later (i.e., 21–26 d after delivery), antigen presentation was assessed in the subcutaneous LNs by injecting the mice with CFSE-labeled OT-I T cells and then visualizing cell proliferation 44 h later by flow cytometry. A shows, for individual mice, the percentage of OT-I cells having undergone more than one division cycle (n = 3 and n = 8–9 mice for B6-mated and Act-mOVA–mated groups, respectively, compiled over three independent experiments), whereas B shows representative CFSE dilution plots. Some postpartum B6-mated females shown in B were also intravenously injected with 100 µg OVA immediately before receiving OT-I cells; the percentage of OT-I cells having undergone more than one division cycle in these mice was not significantly different between the hIgG- and LTβR-Ig–treated subgroups (73.4 ± 5.6% vs. 62.5 ± 13.4%, respectively; mean ± SD; n = 4 mice per group compiled over two independent experiments; P = 0.19).
OVA–anti-OVA immune complexes on LN FDCs (Fig. 5 B) and contrasted with the virtual total loss of OT-I proliferative responses in B6CBAF1 virgin females 7 d after their intravenous injection with 100 µg soluble OVA alone (Fig. S1 A). As with the response to placental OVA, OT-I T cells showed an extended phase of proliferation in the LNs but not the spleen (Fig. S5), in correlation with the rapid disappearance of splenic OVA+ FDC foci (not depicted). As shown in Fig. 5 C, the extended phase of OVA presentation could also be seen in KIR-D− B6CBAF1 mice bearing OVA–anti-OVA immune complexes that were sublethally irradiated and reconstituted with wild-type B6CBAF1 bone marrow as above (see section describing Fig. 2) and, conversely, was reduced in B6CBAF1 mice treated with LTβRIg (Fig. 5 D). As expected (Wu et al., 2000), the loading of OVA–anti-OVA immune complexes onto FDCs was complement dependent, as splenic OVA+ FDC networks were apparent in control B6 but not Cr2−/− mice 2 d after antigen and antibody injection (unpublished data).

Together, these data indicate that the persistent sampling of FDC-bound antigen by bone marrow–derived APCs is a general phenomenon. However, it is notable that our ability to visualize the pathway was confined to mice on a B6CBAF1 background. Thus, although late gestation Act-mOVA–mated B6 mice showed systemic OT-I proliferation and OVA accumulation on FDCs (Fig. 4), antigen did not persist on LN FDC networks in these mice for much longer than 1 wk after delivery, nor did these mice show convincing OT-I proliferation more than ~1–2 wk after delivery (unpublished data), which is in line with previous data (Moldenhauer et al., 2009). Similar data were obtained with Act-mOVA–mated BALB/c females and CD4+ DO11.10 TCR transgenic anti-OVA T cells, as well as with B6 virgins injected with OVA and anti-OVA antibodies (unpublished data). These results are likely explained by the well-known strain-specific differences in complement physiology (Falus et al. [1987] and references therein), which may affect antigen-loading capacities or rates of antigen turnover on the FDC cell surface. Indeed, approximately two times more OVA+ FDC foci per LN cross section were initially formed by equivalent amounts of OVA–anti-OVA immune complexes in B6CBAF1 and CBA/J mice, as compared with B6 and BALB/c mice, and these immune complexes were still clearly visible 3 wk later in B6CBAF1 and CBA/J mice but not in B6 and BALB/c mice (Fig. S6). These strain-specific differences meant that Cr2−/− mice, which were on a B6 background, could be used for demonstrating the complement dependence of antigen loading onto FDCs (e.g., Fig. 4).

Figure 4. Complement and antibody involvement in placental OVA shedding and FDC accumulation. (A) OVA accumulation on FDC networks in complement- and CR1/2-deficient mice. After mating to Act-mOVA males, spleens were collected on E17.5–19.5 from control B6 females (n = 16) or females deficient in various components of the complement system [C1qa−/− [n = 9], C4−/− [n = 8], C3−/− [n = 8], or Cr2−/− [n = 6] mice compiled over at least three independent experiments per group], and tissue sections were stained with anti-OVA antibodies. The mean number of OVA+ FDC networks per splenic cross section is displayed ± SEM. (B) Systemic OVA presentation in complement-, CR1/2-, and B cell–deficient (µMT) mice. Knockout or control B6 females were mated to B6 or Act-mOVA males, and antigen presentation was assessed in the spleen by injecting the mice with CFSE-labeled OT-I T cells on E14.5–19.5. Cell proliferation was then visualized 42–44 h later by flow cytometry. Data are representative of n = 2–9 Act-mOVA–mated mice per group, each from at least two independent experiments.
but not for evaluating the overall complement dependence of persistent antigen presentation.

**LN-resident DCs are the primary cell type that acquires FDC-bound antigen for presentation to CD8 T cells**

Currently, DCs are the only cell type thought to have the capacity to efficiently process exogenous antigens for cross-presentation on MHCII molecules. To confirm that DCs were mediating the presentation of FDC-bound antigen, and to identify the relevant DC subset or subsets, we performed ex vivo antigen presentation assays using various APC populations sorted from the subcutaneous LN of postpartum mice. As shown in Fig. 6A, LN-resident DCs (i.e., the CD11chhi MHCIIint population; see Fig. S7 for our sorting scheme) induced a clear proliferative response in co-cultured OT-I T cells when the DCs were isolated from Act-mOVA–mated but not B6 control–mated females. In contrast, no antigen-specific response was seen when OT-I cells were co-cultured with Langerhans cells, plasmacytoid DCs, or B cells isolated from postpartum Act-mOVA females, and only a low and inconsistent level of antigen-driven proliferation was seen in co-cultures with non–Langerhans cell CD11chhi MHCIIint migratory DCs (Fig. 6A).

Conversely, we assessed whether DCs were required for the extended phase of antigen presentation. These experiments were performed in the context of FDCs loaded with classical antigen–antibody immune complexes and took advantage of CD11c–diptheria toxin (DT) receptor (DTR) transgenic mice, which through their CD11c promoter–directed expression of the DTR render DCs susceptible to DT-induced ablation (Jung et al., 2002). Specifically, we loaded FDCs in Kb−Db− B6CBAF1 females with 100 µg...
OVA–250 µg anti-OVA immune complexes and then 2 d later performed the sublethal irradiation-based protocol described above (see section describing Fig. 2) to transplant them with bone marrow from CD11c-DTR mice. 12 d after transplantation, we started giving half the mice daily intravenous injections of DT and then transferred OT-I T cells 14 d after transplant to detect OVA presentation. As compared with non-DT–treated mice, DT-treated mice showed significantly reduced OT-I proliferation (Fig. 6 B), with the remaining proliferation in some of these mice attributable to the inability of intravenous DT injection to completely deplete all LN-resident DCs (as opposed to splenic DCs; not depicted). Although DT treatment of CD11c-DTR mice is known to affect cell types other than DCs (e.g., Probst et al., 2005), these data, when considered in combination with the results of our ex vivo co-culture experiments, provide strong evidence that LN-resident DCs are the primary, if not exclusive, cell type acquiring antigen from FDC depots for subsequent presentation to CD8 T cells.

Tolerogenic T cell responses to the presentation of FDC-retained OVA, even in the presence of exogenous adjuvants

Lastly, we asked whether the presentation of antigen acquired from FDC depots was tolerogenic or immunogenic. Our first approach involved assessing the 5-d expansion of OT-I T cells transferred into postpartum Act-mOVA–mated females 14–17 d after delivery, when it was likely that virtually all of the presented OVA would have been acquired from FDC-associated depots. To provide positive controls for T cell deletional tolerance and T cell priming, we injected virgin females intravenously on the day of OT-I transfer with endotoxin-free, soluble OVA, with or without the adjuvant combination of agonistic anti-CD40 antibodies and the TLR3 agonist polyinosinic:polycytidylic acid (poly(I:C)). These adjuvants would be expected to convert the poor CD8 T cell response induced by antigen alone to strong T cell priming, even in the absence of CD4 T cell helper function (Ahonen et al., 2004). The dose of injected OVA (10 µg) was chosen because it was the lowest known to give good OT-I proliferative responses (Li et al., 2001); the doses of anti-CD40 antibodies and poly(I:C) (30 µg and 15 µg, respectively) were based on an empirical estimation (unpublished data) of the minimum required to induce good conversion of T cell deletion to T cell priming.

As expected, control virgin females injected with OVA alone showed OT-I proliferation without a significant numerical expansion of the cells (Fig. 7 A, compare CFSE dilution plots with graph), suggesting that proliferation was matched

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**Figure 6.** FDC-associated antigens are presented by LN-resident DCs. (A) Ex vivo co-culture. B6CBAF1 females were mated to either B6 or Act-mOVA males, and 10–21 d after delivery, subcutaneous LNs were collected and respectively pooled for each group of mice. Sorted populations were then co-cultured with CFSE-labeled OT-I cells, and cell proliferation was assessed 90 h later. Representative data are from one of three independent experiments and show results for an APC/T cell ratio of $8 \times 10^3:5 \times 10^4$. (B) Dependence of FDC-associated antigen presentation on CD11c+ APCs. $K^{b-}D^{b-}$ B6CBAF1 virgins were reconstituted with CD11c-DTR bone marrow 2 d after immune complex formation and then given CFSE-labeled OT-I T cells 2 wk later to assess antigen presentation. Cell proliferation in the subcutaneous LNs was visualized 44 h later by flow cytometry. The indicated animals began receiving daily intravenous injections of 100 ng DT starting 2 d before OT-I transfer. Given the high degree of antigen-nonspecific proliferation seen after bone marrow transplantation (see Figs. 2 C and 5 C), we analyzed proliferation as the percentage of OT-I cells having undergone more than two, rather than one, division cycles ($n = 6$ animals per group compiled over two independent experiments).
with concurrent cell deletion. In contrast, adjuvant coinjection induced extensive OT-I proliferation and a mean ~55-fold expansion of the cells (Fig. 7 A) and their expression of IFN-γ (not depicted). A similar pattern was observed using postpartum B6-mated females, which demonstrated that the pathways mediating T cell priming remained generally intact in the postpartum period (Fig. 7 A). Postpartum Act-mOVA–mated female mice also showed OT-I proliferation without significant T cell accumulation, just like control mice injected with OVA in the absence of adjuvants (Fig. 7 A). However, in direct contrast to the response of virgin and B6-mated control mice, the injection of anti-CD40 antibodies plus poly(I:C) into Act-mOVA–mated females did not convert this response to T cell priming, as we failed to detect any consistent numeric expansion of the OT-I cells despite them undergoing more division cycles. Indeed, the fold expansion over nonadjuvant-treated Act-mOVA–mated mice was at maximum only approximately eightfold, and the mean fold expansion was only approximately threefold. Interestingly, several of these mice showed decreased T cell recovery compared with untreated controls.

Together, these results suggested that the presentation of FDC-bound placental OVA was not only inherently tolerogenic but could even induce T cell deletion in the presence of exogenous adjuvants. To substantiate and extend these findings, we used our alternative model of antigen acquisition and presentation based on the loading of FDCs with defined quantities of classical antigen–antibody immune complexes (i.e., 100 µg OVA plus 250 µg anti-OVA antibodies injected into virgin

Figure 7. Tolerogenic T cell responses to FDC-associated antigen. Mice were injected with CD45.1-marked, CFSE-labeled OT-I T cells, and cell proliferation was assessed in the subcutaneous LNs 5 d later. For each experiment, the fold OT-I expansion for each mouse was calculated by normalizing the OT-I/total CD8+ T cell ratio to the mean for this ratio in the virgin, untreated group. The numeric fold differences shown were calculated from the mean fold expansion for each group (bars). The plots showing CFSE dilution versus expression of the activation marker CD62L come from single experiments.

(A) Responses to shed placental OVA. B6- and Act-mOVA–mated females were given OT-I cells 14–17 d after delivery; virgins were processed in parallel. Some mice were injected as indicated with OVA and/or anti-CD40 antibodies (α-CD40) plus poly(I:C) 4–6 h before OT-I transfer. Data are from n = 5–11 mice per group (five independent experiments). The Act-mOVA–mated groups only include mice that showed OT-I proliferation and thus had FDC-retained OVA available for presentation.

(B) Responses to OVA-anti-OVA immune complexes in virgin mice. For groups 1–8, mice were loaded with or without immune complexes and then given OT-I cells 16 d later. Some mice were injected as indicated with α-CD40 antibodies plus poly(I:C) 4–6 h before OT-I transfer and/or with α-CD25 antibodies 12 d after immune complex formation. Groups 9 and 10 were given immune complexes 4–6 h immediately before OT-I transfer. Data are from n = 5–9 animals per group (four independent experiments).
mice). As shown in Fig. 7 B, this model was also associated with OT-I T cell proliferation without a significant degree of cell expansion when the cells were transferred 16 d after immune complex formation (compare group 1 with group 2). Furthermore, injection of anti-CD40 antibodies plus poly(I:C) at the time of T cell transfer not only failed to induce T cell priming but instead induced a dramatic loss of cells (group 4). This extreme phenotype was antigen dependent because OT-I cell numbers remained relatively high in antigen-free mice that were adjuvant injected at the time of T cell transfer, even though the cells underwent some level of bystander proliferation (group 3). The overall pattern of response was unchanged when the mice were given anti-CD25 antibodies to deplete natural T reg cells and any T reg cell potentially induced by OVA exposure during the weeks before OT-I adoptive transfer (groups 5–8).

Importantly, OT-I cells transferred with adjuvant on the same day of immune complex formation underwent robust expansion (29-fold compared with that induced by immune complexes alone; >100-fold compared with untreated controls; Fig. 7 B, groups 9 and 10). In this latter situation, some immune complexes were presumably taken up directly by DCs without first being bound into FDC-associated depots. Lastly, mice given adjuvants at the time of immune complex formation still showed OT-I deletion when the cells were given 16 d later (Fig. 8 B). Together, these data revealed the acquisition and presentation of FDC-bound antigen by DCs to be a remarkably tolerogenic pathway for CD8 T cells that is independent of the physical form of the loaded antigen (membranous placental material versus antigen–antibody immune complexes), T reg cells, and the level of inflammation at the time of initial antigen loading or ultimate TCR engagement.

**DISCUSSION**

Because of the physical separation between B cell follicles and the T cell zones, the function of FDCs has thought to be confined exclusively to the realm of humoral immunity. Contrary to this view, we present evidence in this study that FDC-associated antigen depots are persistently sampled by LN-resident DCs to regulate the T cell immune response. This evidence is founded on the observation that the retention of antigen by LN FDCs correlated with extended phases of antigen presentation to LN CD8 T cells. Functionally, we showed that these extended phases involved continual antigen acquisition by bone marrow–derived APCs and a radioresistant antigen depot. We then showed that the antigen depots were indeed FDC associated and that the APCs were LN-resident DCs. As discussed in the following paragraphs, the pathway links several critical, but poorly understood factors that regulate T cell immune responses, including antigen persistence, complement activation, and the clearance of apoptotic material.

The acquisition and presentation of FDC-bound antigen appears to be a general process because it applies to at least both classical antigen–antibody immune complexes as well as the immune complexes formed from shed placental material. At present, the exact composition of these latter complexes remains undefined. Previously, we showed that OVA accumulation on FDCs during pregnancy did not involve the simple proteolytic release of OVA from the trophoblast cell surface (Erlebacher et al., 2007). Therefore, it is likely that the protein encoded by the Act-mOVA transgene continues to be membrane-associated upon its release from the placenta and that it is some component of this membranous material that mediates complement deposition and FDC binding. Moreover, our demonstration here that shedding was independent of the complement system, antibodies, or NK cells suggests that the process was intrinsic to placental trophoblasts. Analogously, the human placenta is known to release large amounts of subcellular material, including >100-nm-diameter microparticles occasionally containing the nuclear remains of apoptotic syncytiotrophoblasts, as well as the 40–100-nm-diameter multivesicular body–derived particles known as exosomes (Redman and Sargent, 2007). Both apoptotic material and exosomes can be opsonized by complement (Bard et al., 2004; Papp et al., 2008; Paidassi et al., 2009), which in the case of exosomes likely mediates FDC binding (Denzer et al., 2000; Qazi et al., 2009). Because the accumulation of placental OVA on maternal FDCs was apparent as early as embryonic day (E) 11.5, before any substantial T cell response to the placenta (Erlebacher et al., 2007), it is unlikely that the FDC binding of placental OVA requires an induced maternal antibody response to OVA or to any other T-dependent placental antigen. In contrast, it remains possible that natural antibodies with placental reactivity or antiplacental antibodies arising in late gestation contribute to FDC binding.

Although our results provide firm evidence that DCs persistently sample FDC-retained antigen, the exact mechanism of antigen acquisition is currently unclear. Provocatively, the presence of scattered DCs within B cell follicles (Berney et al., 1999; Yu et al., 2002; Lindquist et al., 2004) suggests a short-range process that allows DCs to sample the follicular antigenic environment before migrating to the T cell zones. Interestingly, cognate B cells have recently been shown to acquire FDC-bound immune complexes via a pathway that involves direct cell–cell contact, surface Ig, and capture of CD157, an FDC cell surface marker (Suzuki et al., 2009). Although an analogous pathway might also be available to DCs if mediated by non–Ig receptors, we have found that the surface levels of CD157 on LN DCs, which were rather appreciable in some DC subsets, were not significantly altered after LTBR-Ig treatment (unpublished data). This suggests that DC-associated CD157 is expressed cell-intrinsically rather than acquired from FDCs. Furthermore, direct B cell capture of FDC-retained antigen has been observed as late as 9 d after immune complex formation, when the complexes are mainly condensed into large, ≈0.3-µm-diameter iccosomes (Suzuki et al., 2009). In contrast, immune complexes retained by FDCs for >1 wk become diffusely localized over FDC dendrites and appear to be ingested preferentially by tingible body macrophages (Szakal et al., 1988). It is unknown whether this latter process is more relevant to DCs and whether it involves direct cell–cell contact or the release of antigen from the FDC surface.
and its transient cell-free diffusion. Such diffusion might also allow released antigen to reach the T cell zones. Further work will also be required to elucidate the receptors that mediate antigen uptake by DCs, as they might include the CD11b/CD18 (CR3) and CD11c/CD18 (CR4) complement receptors (which would be relevant to the immune complexes formed from both antibodies as well as membranous material), Fc receptors (which would be relevant only to antibody-containing immune complexes), the scavenger receptor CD36 or the integrins αvβ3 and αvβ5 (which might be relevant only to cases of membrane-associated material), or more antigen-specific receptors (such as the mannose receptor in the case of OVA).

The engagement of CR3 during the acquisition of FDC-bound OVA is a particularly attractive possibility because it might help explain why the presentation of acquired antigen was so tolerogenic. Recent work has demonstrated that DC activation is suppressed upon ligation of CR3 (and to a less well-established extent CR4), even if the cells are also exposed to LPS or CD40 ligand (Verbovetski et al., 2002; Morelli et al., 2003; Sohn et al., 2003; Skoberne et al., 2006; Behrens et al., 2007). This is analogous to our data showing that the presentation of FDC-associated OVA still induced OT-I deletion when the mice were given anti-CD40 antibodies and poly(I:C) at the time of T cell transfer. Paradoxically, these adjuvants even enhanced T cell deletion. However, it is unlikely that ligation of DC CR3 (or CR4) was alone responsible for tolerance induction to FDC-bound OVA because the concurrent injection of adjuvant, immune complexes and OT-I cells induced strong T cell priming, as expected. This result conversely indicates that tolerance was also not being induced solely by a component of the immune complexes themselves. Given that tolerance also showed no requirement for T reg cells, our data instead suggest that DCs acquiring antigen from FDC-associated depots require a factor produced specifically with the follicle if not by the FDC itself to assume a tolerogenic phenotype. It is also possible that the DCs acquiring antigen from FDC-associated depots are intrinsically specialized for tolerance induction.

The tolerogenic sampling of FDC-bound antigen by LN-resident DCs might have implications for any situation involving antigen presentation and complement activation. For example, the pathway might help explain the systemic suppression of antifetal/placental T cell responses observed during pregnancy (Erlebacher et al., 2007), as well as situations in which exogenous antigen–antibody immune complexes are tolerogenic (Mosconi et al. [2010] and references therein). Moreover, the pathway might contribute to the range of CD8 T cell dysfunction (which includes T cell deletion) associated with certain chronic viral infections (Shin and Wherry, 2007). This latter possibility is consistent with the expectation that antiviral antibodies arising during infection would generate FDC-associated viral antigen depots, as well as with our observation that T cells are deleted even under conditions of inflammation. Interestingly, the persistent T cell response to influenza virus antigen observed after live virus clearance has recently been attributed to the sampling of residual antigen depots within the lungs by pulmonary DCs, before their migration to the mediastinal LN (Kim et al., 2010). These depots are radioresistant and have been suggested to be associated with FDCs present within infection-induced tertiary lymphoid structures because LTβR–Ig treatment diminished the number of CD4 T cells proliferating in response to residual influenza antigen. However, antigen was not directly shown to be associated with lung FDCs, and LTβR-Ig treatment also diminished naive anti-influenza CD4 T cell numbers within the mediastinal LN, leading to a relatively unchanged CFSE dilution profile (Kim et al., 2010). This response starkly contrasts with the complete abrogation of antigen presentation we observed in our system. Thus, the role of FDCs in the presentation of residual influenza virus antigen remains to be completely established. It is also unclear how a single pathway would explain the observation that residual influenza antigen presentation can promote T cell memory in the CD4 but not CD8 compartment (Jelley-Gibbs et al., 2005, 2007). Nonetheless, these results together with ours suggest that the sampling of radioresistant antigen depots may have different effects on the quality of the T cell response depending on the type of depot, the subset of sampling DC, and the type of T cell.

We are also struck by the potential relevance of the antigen sampling pathway described in this study to the pathogenesis of systemic lupus erythematosus (SLE), an autoimmune disease characterized by the production of antibodies predominantly toward the nuclear autoantigenes concentrated within the membrane-associated microparticles (i.e., blebs) produced by apoptotic cells (Casciola-Rosen et al., 1994; for review see Manderson et al., 2004). Specifically, our data would suggest that any defect in the ability of DCs to retain immune complexes, or for DCs to acquire these immune complexes, might limit the peripheral deletion of T cells reactive toward the autoantigens associated with complement- or immune complexes, or for DCs to acquire these immune complexes, might limit the peripheral deletion of T cells reactive toward the autoantigens associated with complement-opsonized apoptotic microparticles. The ensuing increase in the number of naive SLE-relevant autoreactive T cells would increase the risk of their activation, which would in turn set the stage for a loss of B cell tolerance. This hypothesis applies to the preclinical stages of the disease and assumes that the constitutive, DC-mediated presentation of apoptotic material acquired from FDCs indeed constitutes a physiologically significant pathway for tolerizing SLE-relevant autoreactive T cells, a postulation which needs experimental verification. However, the hypothesis is consistent both with the SLE predisposition seen in patients with genetic deficiencies in upstream components of the classical complement pathway (i.e., C1q, C2, and C4; for review see Manderson et al., 2004) and the association between SLE susceptibility and polymorphisms in ITGAM, which encodes the α chain (i.e., CD11b component) of CR3 (Moser et al., 2009), as well as with the finding that apoptotic material is often tolerogenic under physiological conditions (Paidassi et al., 2009). Moreover, the hypothesis is consistent with the observation that SLE, while associated with the impaired clearance of apoptotic material (for review see Manderson et al., 2004), tends to be better...
modeled in gene-targeted mice when the induced deficiencies impair both apoptotic cell engulfment as well as FDC function (e.g., as with deficiencies in C1q, C4, Mfge8 [milk fat globule epidermal growth factor 8], or lymphoxygen signaling), rather than apoptotic cell engulfment alone (e.g., as with deficiencies in CD14, CD36, β3 integrin, or β5 integrin; Taylor et al., 2000; Ravichandran and Lorenz, 2007; Kranich et al., 2008).

MATERIALS AND METHODS

Mice. B6CBAF1/J (B6CBAF1), B6, CBA/J, and BALB/c were purchased from The Jackson Laboratory. Act-mOVA (also called CAG-OVA), C3+/−, and μMT mice, all on B6 backgrounds, were purchased from The Jackson Laboratory. OT-I Rag−/−, MHC-I-deficient H-2Kd−/− H-2Db−/− (K−−−− D−−−−), B6 SJL-Pepc−/−BoyAlTc (B6 CD45.1), B6.103−/−, and B15−/− mice were all on B6 backgrounds and were purchased from Taconic. OT-I Rag−/− and K−−−− D−−−− males were mated to CBA/J females to generate B6CBAF1 OT-I and K−−−− D−−−− B6CBAF1 mice, respectively. OT-I Rag−/− mice were also crossed to B6 CD45.1 mice, and their offspring then to CBA/J mice, to generate OT-I CD45.1 B6CBAF1 mice. B6 CD45.1 and CBA/J mice were crossed to generate the CD45.1 B6CBAF1 mice used as bone marrow donors. Cl4−/− (Botto et al., 1998), Cytb−/− (Wesely et al., 1995), C2−/− (Ahearn et al., 1996), and CD11c−/−DTR (Jung et al., 2002) mice, all on B6 backgrounds, have been previously described. Mice were maintained in a specific pathogen-free facility, and all experiments were approved by the New York University School of Medicine Institutional Animal Care and Use Committee. All mating experiments used virgin females; noon of the day of the appearance of a copulation plug was counted as E0.5. Genotyping of Act-mOVA pups was performed as previously described (Erlebacher et al., 2007); only those Act-mOVA−/− mated mice that generated a litter containing at least one Act-mOVA−/− pup were included for analysis. All pups were sacrificed within 3 d after delivery.

Immunohistochemistry. Staining for OVA and CD35 was performed as previously described (Erlebacher et al., 2007). In brief, tissues were fixed overnight at 4°C in fresh 4% paraformaldehyde in PBS and then immediately embedded in paraffin (Paraplast X-TRA; Thermo Fisher Scientific) at 56°C, using a 20-min cycle time. Serial sections, cut at 5 μm, were treated with 1 mg/ml trypsin and then stained with biotinylated rabbit anti-OVA antibodies (1:3,000 dilution; Fitzgerald Industries International, Inc.) or anti-CD35 antibodies (1:100 dilution; clone 53–6.7; BD). Secondary reagents (horseradish peroxidase−conjugated streptavidin or horseradish peroxidase−conjugated anti−rat antibodies, both purchased from Jackson ImmunolResearch Laboratories, Inc.) were then applied followed by biotin−tyramide amplification (PerkinElmer). The sections were then incubated with Alexa Fluor 594− or Alexa Fluor 488−conjugated streptavidin (Invitrogen) and counterstained with DAPI. Microscopy was performed using an Axiosimager M1 (Carl Zeiss, Inc.) and Axiosview software (Carl Zeiss, Inc.). All images within an experiment were equivalently manipulated with Photoshop (Adobe Systems) to enhance brightness and contrast.

T cell adoptive transfers and flow cytometry. OT-I T cells were prepared by magnetic bead−based negative selection (anti-CD4, MHCII, and CD19) on LD columns (Miltenyi Biotec). The B cell−depleted fraction was stained with antibodies to MHCII (clone M5/114.15.2), CD103 (2E7), CD326 (G6.8), and CD11c (HL-3) to identify DC subsets (Henri et al., 2010), whereas the B cell−enriched fraction was stained with antibodies to MHCII and CD19 (6D5) to identify B cells. Sorting was performed on a MoFlo cell sorter (Dako). Serial twofold dilutions of 0.1−32 × 106 DCs were co-cultured with 5 × 105 CFSE−labeled CD45.1 OT-I T cells in V-bottom 96-well dishes as described previously (Botto et al., 1998), pretreated with the FcγR−blocking mAb 2.4G2, and analyzed using a flow cytometer (LSRII; BD) and FCS Express software (De Novo Software). For 44−h adoptive transfer experiments, OT-I T cells were identified as being CD8α−/− (clone 53–6.7) Vα2−/− (B20.1) and CFSE−; for 5−d adoptive transfer experiments they were identified as being CD8α−/− Vα2−/− CD45.1−/− (A20). These latter cells were also analyzed for CD62L expression (MEL−14). Donor DCs were identified after bone marrow transplantation as being CD45.1+ CD19− (6D5) CD11c+ (HL-3) MHCII+ (M5/114.15.2) and were subtyped using antibodies toward CD86 and CD11b (M1/70). Antibodies were obtained from BD or BioLegend.

Hysterectomies. Mice were anesthetized with a ketamine/xylazine/acepromazine cocktail, and a lower abdominal transverse incision was made to expose the uterus. The uterus was removed after ligating the cervical base and the cranial end of each uterine horn with Coated Vicryl 5-0 sutures (Ethicon). The abdominal wall was sutured closed, and the skin incision was closed with wound clips. Sham operations duplicated this protocol excluding ligation and removal of the uterus.

Bone marrow transplantation. Mice were irradiated with 600 cGy cesium-137−sourced γ-radiation (Gammarcell 40; MDS Nordion) and then immediately injected intravenously with 4−× 106 congenerically marked CD45.1 B6CBAF1 or CD11c−/−DTR bone marrow cells. The mice were concomitantly injected intraperitoneally with 250 μg anti-NK1.1 NK cell−depleting antibodies (PK136; Bio X Cell) and 2 d later with 200 μg/kg cyclophosphamide (Sigma-Aldrich), as adapted from previous protocols (Shamsi et al., 1990; Tomita et al., 2000). The mice were given water supplemented with 1% Sulfatrium antibiotics for the remainder of the experiment and were injected with 10μg CFSE−labeled OT-I T cells 2 wk after transplantation. OT-I T cells were not rejected in mice with high levels of chimerism, as expected. For the control experiment involving untransplanted mice (Fig. 2 A), it was necessary to give the mice 250 μg anti-NK1.1 antibodies at the time of T cell transfer, in addition to an increased number of transferred cells (3 × 106), so that appreciable numbers of OT-I T cells would remain for analysis 2 d later.

Other reagents, immune complex generation, LTβR−lg−mediated FDC depletion, DT−mediated DC depletion, and T reg cell depletion. OVA (grade VI; Sigma-Aldrich) and rabbit anti-OVA (Fitzgerald Industries International) were applied to Detoxi-Gel columns (Thermo Fisher Scientific) to reduce endotoxin levels to <0.012 endotoxin unit per mg, as determined by the Limulus Amebocyte Lysate test (BioWhittaker). All OVA−anti-OVA immune complexes were formed in vivo via intravenous injection of 250 μg anti-OVA antibodies immediately followed by intravenous injection of 100 μg OVA. Anti-CD40 antibodies were obtained from Bio X Cell, and poly(I-C) was obtained from GE Healthcare The mouse LTβR−human Ig (LTβR−Ig fusion reagent was the gift of J. Browning (Bigen Idec, Weston, MA), and control polyconal human IgG was obtained from MP Biomedicals. Both of these reagents were intravenously injected at 100 μg, 100 ng DT (EMD) was given daily for 4 d by intravenous injection. T reg cells were depleted in vivo by intravenous injection of 500 μg anti-CD25 antibodies (PC61; Bio X Cell) 4 d before OT-I transfer. Depletion was confirmed at sacrifice by flow cytometry of CD4+ (clone RM4-5) CD25+ (clone 7D4) cells.

DC sorting and ex vivo co-culture with T cells. LN cell suspensions were prepared by enzymatic digestion as previously described (Erlebacher et al., 2007), pretreated with the FcγR−blocking mAb 2.4G2, and then separated into B cell−enriched versus B cell−depleted fractions by magnetic bead−based negative selection (CD19) on LD columns (Miltenyi Biotec). The B cell−depleted fraction was stained with antibodies to MHCII (clone M5/114.15.2), CD103 (2E7), CD326 (G6.8), and CD11c (HL-3) to identify DC subsets (Henri et al., 2010), whereas the B cell−enriched fraction was stained with antibodies to MHCII and CD19 (6D5) to identify B cells. Sorting was performed on a MoFlo cell sorter (Dako). Serial twofold dilutions of 0.1−32 × 106 DCs were co-cultured with 5 × 105 CFSE−labeled CD45.1 OT-I T cells in V-bottom 96-well dishes as described previously (Belz, 2010). After 90 h, the T cells were visualized by flow cytometry as the CD8α−/− Vα2−/− CD45.1−/− population.

Statistical analysis. The percentage of OT-I T cells engaged in antigen−driven proliferation in different postpartum Act-mOVA mice did not fall into a normal distribution, as some mice (approximately one out of five) did not
show a detectable response despite having act-mOVA\textsuperscript{+} pups. Thus, a two-tailed Mann-Whitney test was used for all comparisons involving OT-I proliferation in Act-mOVA-mated females, including the fold expansion data shown in Fig. 7 A. For consistency, a two-tailed Mann-Whitney test was also used to analyze the data in Fig. 7 B. All other comparisons were performed using a two-tailed Student's t test. P < 0.05 was taken as the cutoff for statistical significance.

**Online supplemental material.** Fig. S1 shows the kinetics of OVA presentation after the intravenous injection of soluble protein into virgin and B6-mated postpartum mice and the maintenance of persistent of OVA presentation and FDC-bound OVA deposits in Act-mOVA-mated mice subjected to hysterectomy in the immediate postpartum period. Fig. S2 demonstrates OVA retention on maternal FDCs and the appearance of donor-derived DCs according to application of the bone marrow transfer protocol used to generate the data shown in Fig. 2. Fig. S3 shows recovery of FDC networks but loss of their antigen deposits 3 wk after a single injection of LTβR-Ig. Fig. S4 shows that placental antigens are shed and presented to T cells in mice deficient in perforin and NK cells. Fig. S5 shows that the formation of OVA-anti-OVA immune complexes leads to an extended phase of OVA presentation in the subcutaneous LN but not the spleen. Fig. S6 illustrates differential loading and retention of OVA on LN FDCs in various mouse strains after the formation of OVA-anti-OVA immune complexes. Fig. S7 illustrates the gating scheme used for the sorting experiment of Fig. 6 A. Fig. S8 demonstrates that OT-I T cells do not expand when transferred 3 wk after OVA-anti-OVA immune complexes were formed in the presence of adjuvants. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100354/DC1.

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