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Preferential Infection of Immature Dendritic Cells and B Cells by Mouse Mammary Tumor Virus

Sonia Vacheron, Sanjiv A. Luther, and Hans Acha-Orbea

Until now it was thought that the retrovirus mouse mammary tumor virus preferentially infects B cells, which thereafter proliferate and differentiate due to superantigen-mediated T cell help. We describe in this study that dendritic cells are infectable at levels comparable to B cells in the first days after virus injection. Moreover, IgM knockout mice have chronically deleted superantigen-reactive T cells after MMTV injection, indicating that superantigen presentation by dendritic cells is sufficient for T cell deletion. In both subsets initially only few cells were infected, but there was an exponential increase in numbers of infected B cells due to superantigen-mediated T cell help, explaining that at the peak of the response infection is almost exclusively found in B cells.

The level of infection in vivo was below 1 in 1000 dendritic cells or B cells. Infection levels in freshly isolated dendritic cells from spleen, Langerhans cells from skin, or bone marrow-derived dendritic cells were compared in an in vitro infection assay. Immature dendritic cells such as Langerhans cells or bone marrow-derived dendritic cells were infected 10- to 30-fold more efficiently than mature splenic dendritic cells. Bone marrow-derived dendritic cells carrying an endogenous mouse mammary tumor virus superantigen were highly efficient at inducing a superantigen response in vivo. These results highlight the importance of professional APC and efficient T cell priming for the establishment of a persistent infection by mouse mammary tumor virus. The Journal of Immunology, 2002, 168: 3470–3476.

Mouse mammary tumor virus (MMTV) is a retrovirus that has been shown to preferentially infect B lymphocytes (1, 2). It has developed a strategy that requires a strong immune response to allow chronic infection of the host and completion of the viral life cycle (Refs. 3 and 4 and reviewed in Ref. 5). After infection, B cells present a superantigen (SAg) on their cell surface, which is presented in the context of MHC class II, and leads to SAg-mediated T cell help and B cell differentiation. Therefore, in the draining lymph node (LN) the majority of the amplification is due to preferential cell division of SAg-presenting infected B cells even though infected lymphocytes can produce infectious virus particles (6, 7). Peak responses are observed between days 4 and 6, when up to one-third of the B cells in the draining LN are infected and T cells expressing SAg-reactive TCR Vβ element have expanded (8). Nursing of B cell-deficient mice by virus-infected mothers has indicated that the viral life cycle is interrupted in the absence of B cells (1).

Optimal priming of an immune response is required for generation of a chronic efficient immune response. Interestingly, a chronic immune response is installed in the LN draining the site of injection. This chronic reaction is sustained by continuous SAg presentation (9). Thereafter the SAg-reactive T cells are preferentially lost from nondraining secondary lymphoid organs by a slow deletion of SAg-reactive T cells. Overall, the SAg-mediated immune response in the draining LN is very similar to classical immune responses despite the action of SAg and systemic deletion of SAg-reactive T cells. This similarity was surprising given that MMTV SAg is believed to be presented by B lymphocytes, which are inefficient at priming immune responses in vivo. It was previously observed that T cell priming after MMTV infection occurred in close proximity of dendritic cells (DC) in the paracortex of the draining LN (9). In addition, it was shown that DC can prime the MMTV SAg response (10). No evidence, however, was presented to indicate whether DC are infected by MMTV.

DC are professional APC capable of inducing primary immune responses in lymphoid organs (11). In peripheral tissues that are prone to Ag encounter, such as skin, mucosa, and Peyer’s patches, immature DC are present with a strong ability to take up Ags and a weak Ag presentation capacity (12). After exposure to foreign Ags in an inflamed environment, nonlymphoid DC migrate through lymphatics to the T cell area of draining lymphoid organs, become highly efficient in Ag presentation, and prime naïve T cells (11, 12). DC within the epidermis called Langerhans cells (LC), for example, are directly exposed to skin injury (for review see Ref. 11). DC present in these sites have been shown to be early targets of viruses such as HIV (13, 14) and other viruses (15, 16).

Because optimal priming is required to induce an efficient long-lasting immune response to classical Ags we analyzed the infection status of DC after MMTV infection. Immature DC were infected at least as well as B lymphocytes and led to SAg presentation. These results show that before SAg-mediated amplification DC and B cells are infected to similar levels and suggest a key role of DC in the priming of the MMTV SAg response in vivo.

Materials and Methods

Mice and virus injection

BALB/c mice were purchased from Harlan Olac (Bicester, U.K.). BALB.D2 mice were obtained from Dr. H. Festenstein (London, U.K.) and BALB.D2 mice were obtained from Harlan Olac (Bicester, U.K.). BALB.D2 mice were obtained from Dr. H. Festenstein (London, U.K.) and

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"Abbreviations used in this paper: MMTV, mouse mammary tumor virus; DC, dendritic cell; LC, Langerhans cell; LN, lymph node; SAg, superantigen.

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maintained in the animal facilities at the Ludwig Institute (Epalinges, Switzerland) (17). μMT−/− mice were obtained from Prof. K. Rajewski (Cologne, Germany) (18). All mice used were 6–12 wk old. Diluted milk containing MMTV(SW) (∼10⁸ viral particles) was injected into the hind footpad as previously described (19).

Antibodies

For flow cytometry analysis, the following mAb were used: 2.4.G2 (anti-CD16/32; BD Pharmingen, San Diego, CA), Ly-5-FITC (anti-B220; Caltag Laboratories, San Francisco, CA), 17A2 (anti-CD3; BD PharMingen), or CDc6-PE (anti-Mac-1; Caltag Laboratories), biotinylated N418 or HL3-PE (anti-CD11c; BD Pharmingen), 2G9 (anti-MHC class II-PE; BD Pharmingen), 44.22.1 (anti-Vß6) (21), L3T4-PE (anti-CD4; Boehringer Mannheim, Mannheim, Germany), and 53.5-8 (anti-CD8; BD Pharmingen).

DC isolation and culture

LN and spleens were cut into small fragments and digested for 30 min at 37°C in collagenase D (1 mg/ml; Boehringer Mannheim). Aggregates were disrupted by a 5-min incubation in RPMI 1640 (Life Technologies, Grand Island, NY) containing 5 mM EDTA at room temperature. Spleen cells were then either separated on a dense BSA gradient to obtain the DC-rich low-density fraction as described (22) or incubated for 1–2 h at 37°C to enrich DC by adherence.

Bone marrow suspensions were flushed out from bones using a syringe and a 25-gauge needle. Cell suspension was then depleted of MHC class II+ and B220+ cells using rabbit complement and mAb anti-MHC class II M5/114,115.2 (TIB-120) plus anti-rat κ light chain MAR 18.5 (TIB-216) and anti-B220 RA3-3A1/6.1 (TIB-146). Cells (∼10×10⁶/well) were cultured for 6–7 days in six-well plates (Costar, Cambridge, MA) in RPMI 1640 containing 10% FCS and antibiotics and complemented with rGM-CSF at 20 ng/ml (a gift from Immunex, Seattle, WA). The recovery of bone marrow-derived CD11c+ DC was at least 50–60% of the total cultured cells after 1 wk in GM-CSF.

LC were isolated from the ear skin as previously described (23). Briefly, the ears were split into dorsal and ventral parts and digested at 37°C for 20 min with 0.5% trypsin for the dorsal sheets and 40 min with 1% trypsin for the ventral sheets. The epidermis of each part was then peeled off and incubated on HBSS 5% FCS to release LC. For in vitro infection, the different DC preparations were cultured for 24 h in RPMI 1640 10% FCS containing antibiotics and 20 ng/ml rGM-CSF in the presence of MMTV(SW) for 24 h in the hanging drop system as previously described (24).

Where indicated the different populations were sorted by FACS. B and T cells were sorted with anti-B220, anti-CD4, and anti-CD8 Abs. To avoid B cell contamination in DC purifications, B220+ cells were first electronically gated out and the different DC populations were sorted using anti-CD11c and anti-CD11b Abs. B cell contamination after DC sorting was always <1% upon reanalysis. Purities of the analyzed populations are indicated in the figures.

Polymerase chain reaction

DNA (250 ng, which correspond to ∼5×10⁶ cells counted by flow cytometry) extracted from ex vivo sorted cells or from DC populations infected in vitro with MMTV(SW) was amplified by PCR. The primers used were 5’ SW1 (GGCGAACCAGGGACTTATAGG) and 3’ SW2 (GGGAC CCCCCATGAGTATATTTC) specific for mtn-7/MMTV(SW) orf; or 5’ ORF-100 (CTCGAGGAGAAAAGACGACAT) and 3’ VJ71 (CCTCAGG AAAAACTGCGGACTTG) amplifying endogenous mtn-6, -7, -8 and DC by adv/MMTV(SW). PCR conditions were as follows: 1 min at 64°C (for SW1 and 2) or 55°C (for VJ71 and ORF-100), then for both 1 min at 72°C and 1 min at 95°C for 32 cycles in PCR buffer containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 2 mM dNTP, and for radioactive PCR 3 μCi [α-32P]-dATP and 0.5 U Taq polymerase (PerkinElmer/Cetus, Norwalk, CT) on a Biometra cycler. Half of the PCR product was boiled and size-fractionated on a 6% denaturing acrylamide gel.

Cell transfers

Mice were injected either i.p. or s.c. into the hind footpad with the indicated number of BALB.B2 cells in 200 or 20 μl of PBS, respectively. Draining popliteal LNs were removed at day 2–3 to measure the SAg response. Alternatively, PBLs were isolated from heparinized peripheral blood to evaluate the deletion kinetics.

Results

Peripheral deletion of SAg-reactive CD4⁺ T cells is induced in the absence of B cells

To determine whether B cells are essential in the SAg response induced by MMTV, we injected the virus into the hind footpad of B cell-deficient mice (μMT−/−). We examined the percentage of reactive CD4⁺ T cells at day 5, which is the peak of the SAg response. No T cell stimulation could be detected in μMT−/− mice where the SAg-reactive Vß6+ CD4⁺ T cells stayed at 8.5 ± 0.2% in the draining LN cells, whereas in the littermate control mice Vß6+ CD4⁺ T cells increased to 22.6 ± 2.3% (Fig. 1A). Furthermore, the expression of activation markers such as L-selectin on SAg-reactive CD4⁺ T cells remained unchanged during the first 4–6 days after injection in μMT−/− mice (12.8 ± 0.5% of Vß6⁺ CD4⁺ T cells), while in μMT+/− mice the percentage of Mel-14⁺ increased to 48.4 ± 1.7% of Vß6⁺ CD4⁺ T cells (Fig. 1B). According to that, up-regulation of CD69 was also observed in Vß6⁺ CD4⁺ T cells of the littermate mice only (data not shown). In parallel, we used a 100-fold more sensitive assay (7) by following the kinetics of deletion in PBL of injected μMT−/− mice. Surprisingly, we observed a progressive decrease in the number of SAg-reactive Vß6⁺ CD4⁺ T cells in μMT−/− as in littermate control mice (Fig. 1, C and D). These results suggest that, in the absence of B cells, the SAg molecules were presented by other APCs, which induced a slow but efficient peripheral deletion of SAg-reactive T cells.

DC present in the draining LN are infected by MMTV

Based on the observations that after MMTV infection chronic immune responses are installed in the draining LN (9) we addressed the question of whether DC are targets for MMTV infection in vivo.
addition to B cells. We isolated DC populations, B cells, and T cells of the draining popliteal LN at day 2.5 after s.c. injection of MMTV(SW) (Fig. 2). B and T cells before and after sorting are shown in Fig. 2A. CD11c⁺ DC obtained by collagenase digestion represented 7–8% of the total LN, among which 2.4% expressed also the CD11b marker (Fig. 2B). This percentage represented a total of 3–4 × 10⁵ DC per LN, which is 20–40-fold the number of DC found in a naive popliteal LN, where CD11c⁺ DC represented only 1–2% of total cells (data not shown). CD11c⁺CD11b⁻ and CD11c⁺CD11b⁺ populations might represent DC at distinct stages of maturation as previously described for spleen DC isolated from mice treated with the hematopoietic growth factor Flt3L (25, 26). Because highly pure populations could be obtained by cell sorting using flow cytometry, a sensitive PCR assay to detect viral reverse-transcribed DNA in host cells was performed. For quantitation, serial dilutions of BALB/cD2 DNA (containing two copies of mtv-7 per cell) in a constant amount of BALB/c DNA were analyzed. As expected, a clear PCR signal was detected in B cells (Fig. 2C), confirming a previous report (4). But, more interestingly, DC were also infected (Fig. 2C), and both CD11c⁺CD11b⁻ and CD11c⁺CD11b⁺ DC populations exhibited retroviral cDNA, which represented 6–20 copies per 10⁵ cells. This means that both DC subsets are infected to a similar extent and that a minimum of 24–80 DC were infected in the draining LN at day 2.5 (5 × 10⁶ cells in total; 4 × 10⁵ DC). To exclude that the PCR signal in DC was due to a contamination with B cells, we titrated purified B cells isolated from the same LN to BALB/c DNA. The contamination with B cells was <1% as assessed by flow cytometry after DC purification (data not shown). Even the PCR signal obtained after adding 5% B cells was still inferior to the DC signals (Fig. 2C), indicating that the signal obtained in DC was not due to contamination with B cells. Infection levels were quantitated using the internal controls shown in Fig. 2D.

Immature DC populations efficiently take up MMTV in vitro

The DC presenting new Ags are migratory cells entering via the afferent lymphatics. These DC are thought to be derived from LC present in the skin that have been stimulated to migrate (11). We further investigated different DC types to assess whether their maturation stage could be correlated with susceptibility to retroviral infection. Splenocytes were infected during 24 h in vitro, and thereafter DC, B cells, or T cells were purified by FACS sorting (Fig. 3A). As described (27, 28), most DC expressed CD4 or CD8 on the cell surface. However, after a 24-h culture this expression was down-modulated on most DC (data not shown). Twenty-four hours after in vitro infection, mature splenic CD11c⁺ DC, B220⁺ B cells, and CD4/8⁺ T cells were sorted by flow cytometry (Fig. 3A). B cells were infected with 20–60 viral copies per 10⁵ cells. DC exhibited a low viral load of 6–20 copies per 10⁵ cells (Fig. 3D). When immature LC bearing high levels of MHC class II molecules were isolated from epidermal cells (Fig. 3B) submitted to MMTV infection, 60–200 copies per 10⁵ cells were found (Fig. 3E). As a third DC type, immature bone marrow-derived DC were obtained after 5 days of culture in GM-CSF (Fig. 3C). These DC derive from MHC class II-negative progenitors in the bone marrow (29, 30). The maturation of these cells in culture resulted in DC expressing high levels of CD11c and a gradual level of MHC class II molecules, the more mature DC having the highest level of MHC class II molecules. However, both populations were immature, because overnight incubation with LPS induced further maturation as measured by CD40 and CD86 induction and did not show detectable B cell contamination (data not shown). If the virus was added at the onset of the culture, infection was most prominent in the CD11c⁺MHC class IIhigh DC subset after 5 days of expansion (Fig. 3F). This would suggest that DC were infected when they were still at an immature stage (see Table I). The level of infection was ~60–200 copies per 10⁵ cells and was comparable to LC.

Priming of naive SAg-reactive CD4⁺ T cells by DC expressing endogenous SAg

Because DC are infected during the early phase of viral entry in the body (see above), and because they were visualized in close contact with proliferating Vβ6⁺CD4⁺ T cells (9), we wanted to test the efficiency of T cell priming by DC presenting a viral SAg. A large number of CD11c⁺MHC class IIhigh DC was obtained from culture of bone marrow derived from BALB.D2 in GM-CSF. BALB.D2 expresses an endogenous Vβ6-specific SAg. Splenic B220⁺ B cells and bone marrow-derived DC were highly purified by flow cytometry and injected s.c. into the hind footpad of congenic recipient BALB/c mice. To determine the activation state of the responsive T cell subset in draining LNs 36 h after transfer the expression of the early activation marker CD69 was assessed. Interestingly, DC induced up-regulation of CD69 in Vβ6⁺CD4⁺ T cells in a dose-dependent manner, suggesting efficient migration to

![FIGURE 2](http://www.jimmunol.org/Downloaded from http://www.jimmunol.org) Detection of proviral DNA in DC after in vivo infection. Draining popliteal LN of MMTV-injected BALB/c mice were taken on day 2.5. Cell types were purified by flow cytometry according to CD11c and CD11b expression for DC, B220 for B cells, and CD4/8 for T cells. In A (B and T panels) and B (dendritic cells) the unsorted populations are shown in the left panels and the two sorted populations are shown in the middle and right panels, respectively. The PCR results are shown for infection levels of the indicated cell populations in C, D. The PCR quantitation is shown using different BALB.D2:BALB/c ratios. The percentage of each cell type among the total LN cells is indicated on the FACS profiles. B cells, B220⁺; T cells, CD4/8⁺; DC, CD11c⁺CD11b⁻ and CD11c⁺CD11b⁺. This experiment was repeated four times with similar results.
The draining LN (Fig. 4A). The basal level of CD69 expression in noninjected animals was 12.3 ± 2.7%. As few as 6 × 10^4 DC were sufficient to induce up-regulation of CD69 on Vβ6^+ CD4^+ T cells (25.1 ± 3.9% CD69^+ Vβ6^+ CD4^+) while at least 1.5 × 10^6 B cells from BALB.D2 mice were required to activate T cells above the basal level (32.2 ± 5.6% CD69^+ Vβ6^+ CD4^+). Therefore, by comparison to B cells, DC appeared 10- to 25-fold more efficient in triggering SAg-reactive T cells. However, the percentage of Vβ6^+ CD4^+ T cells was only increased to 16.1 ± 2.6% for DC and to 16.5 ± 1.5% for B cells compared with 10.8 ± 0.8% in control animals 2.5 days after injection (Fig. 4B). A strong expansion of reactive T cells was generated only if 1.5 × 10^6 (containing 9 × 10^5 B cells and 1.5 × 10^5 DC) unfractionated splenocytes were injected (30.9 ± 3.7% Vβ6^+ CD4^+ T cells). However, 10 times fewer splenocytes (1.5 × 10^5) were neither able to up-regulate CD69 on Vβ6^+ CD4^+ T cells nor able to induce significant T cell expansion (data not shown). These results suggest a synergistic effect between DC and B cells when they are co-injected.

Clonal deletion of reactive CD4^+ T cells from the periphery after transfer of DC expressing SAg from an endogenous virus

To characterize the role of LC in MMTV infection, immature LC from BALB.D2 mice expressing endogenous SAg were injected into BALB/c mice. We wanted to investigate whether these particular cells were able to 1) migrate from the site of injection in the skin to secondary lymphoid organs, and 2) present SAg to responsive T cells and can clonally delete these responding T cells from the repertoire. The peripheral deletion occurring in the Vβ6^+ CD4^+ T cell subset was measured and compared with animals injected with purified B220^+ B cells and total spleen cells. Importantly, LC induced a significantly faster and stronger deletion than spleen or B cells (Fig. 5). Results on footpad as well as i.p. injection are shown. Intraperitoneal injection was more efficient to mediate deletion of SAg-reactive T cells than footpad injection. After i.p. injection as few as 1600 LC could delete 50% of the SAg-reactive T cells pool in 6 wk (Fig. 5). A strong deletion of Vβ6^+ CD4^+ T cells was obtained after transfer of 2 × 10^5 LC, whereas transfer of 10^6 B cells was clearly less efficient. The transfer of unfractionated splenocytes gave efficient deletion curves, as previously reported (31). This experiment and the data presented on SAg-induced activation of reactive CD4^+ T cells in Fig. 4 suggest that LC can migrate to the T zone of the draining LN and prime and efficiently delete the specific Vβ6^+ CD4^+ T cell subset from the periphery.

Discussion

DC are professional APC able to induce primary T cell responses (11). Therefore, it is not surprising that DC have been described in many systems to either take up viral Ags efficiently or be preferable targets for viral infections. On one side, this allows the immune system to mount an efficient antiviral immune response and

**Table I. Infection level comparison between in vitro and in vivo primary retroviral infection**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Origin</th>
<th>In vivo</th>
<th>In vitro</th>
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<tbody>
<tr>
<td>Immature DC</td>
<td>Skin (LC)</td>
<td>ND</td>
<td>60–200</td>
</tr>
<tr>
<td>Immature DC</td>
<td>Bone marrow</td>
<td>ND</td>
<td>60–200</td>
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<td>Mature DC</td>
<td>Spleen</td>
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<td>ND</td>
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<tr>
<td>B cells</td>
<td>Spleen or LN</td>
<td>2–60</td>
<td>20–60</td>
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* BALB/c mice were injected s.c. with MMTV into the hind footpad and on day 2.5 the draining LN cells were highly purified to get B cells (B220^+) and DC (CD11c^+). Splenic B cells and diverse DC subsets, which are LC (MHC class II^+), bone marrow DC (CD11c^- MHC class II^+), and splenic DC (CD11c^+), were submitted to the retrovirus for 24 h in an in vitro infection system. PCR analysis was performed on the DNA extracted from each cell type using virus-specific primers as described in Materials and Methods. Data are represented as the number of viral copies detected per 10^5 cells, based on the mrv-7^-mrv-7^ standard.
potentially lead to virus clearance (32, 33). On the other side, pathogens can use DC infection to achieve a selective advantage by either impairing Ag presentation by DC or, alternatively, using these cells as a reservoir that helps spread infection to cells present within secondary lymphoid organs (13, 34–36).

It was previously assumed that MMTV preferentially infects B cells and that the SAg presentation of these B cells leads to a strong SAg response. In contrast, it was known that MMTV induces a chronic immune response in the draining LN. Usually such chronic immune responses are initiated only after Ag priming of naïve T cells by DC. Entry into cell cycle was shown to occur like classical Ag responses in the paracortex of the draining LN in close contact to DC (9). In addition, using transgenic mice expressing MHC class II I-E exclusively on DC allowed priming of the SAg response, whereas in mice not expressing this I-E transgene no priming was observed (10). In this study we showed that DC are targets of MMTV infection and that immature DC are infected much more efficiently. These observations explain the induction of the chronic immune response in the draining LN.

A possible feature related to the DC maturation stage could account for the different infection efficiency of various DC populations by MMTV. Immature DC in nonlymphoid tissues can phagocytose and process foreign Ags, a capacity that is progressively lost when the cells are put in culture (37). Fresh LC, but neither cultured LC nor spleen DC, have been shown to process native proteins efficiently for presentation to Ag-specific T cells (38, 39). Proliferating DC progenitors in the bone marrow were also shown to phagocytose particles such as bacillus Calmette-Guérin mycobacteria, whereas the nondividing progeny are weak or inactive (40).

These observations have led to the conclusion that endocytosis is highly regulated during DC life and depends on the maturation stage and on specific stimulations. Different mechanisms possibly used by DC for soluble protein uptake comprise 1) coated pits (41), 2) macropinocytosis, or 3) Birbeck granule pathways (42). DC may likewise acquire retroviral particles via receptor-mediated uptake, a mechanism illustrated by adsorption of dye-labeled dextran to mannose receptor (43), or to DEC-205 receptor (44), both of which are C-type lectin receptors expressed on DC. MMTV particles are composed of glycoproteins, which could potentially interact with lectin receptors at the DC surface.

FIGURE 4. Activation and amplification of SAg-reactive CD4⁺ T cells in the draining LN of BALB/c mice injected with mtv-7-expressing APC. Day 7 bone marrow-derived DC isolated from BALB.D2 mice were FACS sorted according to CD11c⁺MHC class II⁺ expression and transferred in 20 μl PBS into the hind footpad of congenic BALB/c mice. FACS-purified splenic B cells (B220⁺) and T cells (CD3⁺) were transferred in parallel for comparison. The draining popliteal LN was removed and the percentage of CD69⁺ among Vβ6⁺CD4⁺ T cells after 2.5 days (B), was measured by flow cytometry. Individual measures are shown. The curve represents the mean and the SDs are indicated in the text. DC (○) are shown in the left panels; B cells (○) are shown in the right panels. Horizontal gray bars represent the basal levels of Vβ6⁺CD4⁺ T cells and CD69 expression in uninjected control mice.

FIGURE 5. Peripheral deletion kinetics induced by transfer of mtv-7-expressing APCs into congenic BALB/c mice. Epidermal cells isolated from BALB.D2 mice ear skin as in Materials and Methods were cultured overnight in 20 ng/ml GM-CSF to release LC from keratinocytes and enrich them up to 10–15% of the total cells. Titrated doses of mtv-7⁺ splenocytes (left panels), LC (middle panels), and splenic B (right panels) cells purified by flow cytometry were injected into the footpad (A) or i.p. (B) into congenic BALB/c mice. The percentage of SAg-reactive Vβ6⁺CD4⁺ T cells in PBLs was assessed by flow cytometry at the indicated time points. Each curve represents the mean of two mice.
The deletion of SAg-reactive T cells in B cell-deficient mice after s.c. injection is in striking contrast to the results obtained by foster nursing to infected mothers, where no deletion and infection was observed (1). Several explanations might account for this discrepancy. Little is known about differences in Peyer’s patch architecture or viral uptake in B cell-deficient mice during the first 2 wk of life in the gut-associated lymphoid tissue. In addition, it is not known whether differences in DC subsets in these locations exist, and T cells have been shown to be less responsive in such mice. We observed slow deletion kinetics in adult B cell-deficient mice infected s.c. with MMTV using optimal virus doses. It appears likely that higher threshold virus doses are required for induction of a chronic MMTV infection in the absence of B cells. Most likely only a small number of MMTV particles initially infect the neonatal gut epithelium, not reaching infection levels sufficient for an amplification of infection of SAg-reactive T cells to occur.

Immature DC have been described to take up Ag in tissues like skin or mucosa and migrate to secondary lymphoid organs (45–48). During migration, DC progressively mature to become highly immunostimulatory while up-regulating MHC class II and accessory molecules for T cell adhesion and costimulation (11). Such low levels of DC infection have been described previously for other viruses (49–52).

In HIV infection, the role of DC has been extensively studied. Immature DC represent the earliest cell type exposed to infection in vivo due to their particular distribution as sentinel cells within peripheral tissues like skin, blood, and mucosa. Immature DC are preferentially infected by HIV (48). Mature DC can be infected but do not proceed to efficient complete reverse transcription. In vitro exposure to HIV-1 leads to a low level of proviral DNA in DC (53), but the cells promote extensive viral replication only upon interaction with T cells (14). To obtain this increase in virus production and infection levels, both T cells and DC need to express the HIV receptors CD4 and CCR5. In addition, a DC-specific molecule, DC-SIGN, has been implicated in allowing transport of HIV to the draining LN in the absence of DC infection, thus facilitating T cell infection (13).

Besides being an initial source of infectious particles, DC can induce antiviral immunity such as antiviral CTL responses (33, 54–57). Other viruses, such as Rauscher leukemia virus (58) or measles virus (34, 35), have been shown to suppress CTL priming after infection of DC. In the course of MMTV infection, however, no CTL activity was detectable (59). The high frequency of SAg-reactive DC4 T cells may out-compete the SAg-reactive CD8 cells in the priming reaction.

A previous report has shown that thymic DC can express functional endogenous mntl SAg and are able to induce deletion of SAg-reactive thymocytes in vitro (60). However, other studies failed to show efficient priming of SAG responses with DC populations in vitro (61–63). Therefore, we assessed SAG presentation by DC in vivo by transferring DC (or other APC) expressing the endogenous *Mtv*-7 SAg into congenic recipients. Specific T cell activation and amplification could be triggered by bone marrow-derived DC as well as B cells. However, immature LC were the most potent APC, with as little as 1–2 × 10^3 transfused cells leading to efficient T cell deletion. Taken together, these results attest that DC can migrate in the LN draining the site of injection to present SAg molecules and that immature DC can, after transfer into a recipient mouse, up-regulate their immunostimulatory capacity to become highly powerful in peripheral tolerance induction. Curiously, we found that DC purified from the spleen were quite inefficient inducers of peripheral deletion (data not shown). This observation could explain in part the discrepancies between the different studies. It is in agreement with a report where injection of mature splenic DC induce anergy rather than deletion in the thymic Vβ6 subset (64). Moreover, Mazda et al. (65) related that splenic B cells or DC alone are unable to delete Mls-reactive thymocytes. In contrast, a study using a transgenic mouse in which I-E expression is targeted to DC could show that negative selection is achieved by DC in the thymus (66).

We consistently detected a weak infection in dendritic epidermal T cells. These cells originate in the thymus and have a biological relationship with epidermal cells (67, 68). MMTV has been shown to infect B cells, epithelial cells, and DC, as well as weakly dendritic epidermal T cells. The latter was only shown in vitro under conditions where, e.g., T cells are not infected. These results suggest that dendritic epidermal T cells may express the elusive MMTV receptor(s).

In mice lacking mature B cells, DC infection was observed, but no significant T cell priming was detected in the draining LN. This can be due to the low frequency of infected DC in the draining LN and the fact that, contrary to B cells, numbers of infected DC do not increase during T cell interaction.

Upon MMTV injection, the number of CD11c^+ DC found in the draining popliteal LN was surprisingly high (3–4 × 10^5). We hypothesize that s.c. injection of virus may also induce emigration of DC from skin or immigration of DC into the LN via high endothelial venules. It has been proposed that, as for epidermal LC, DC in the afferent lymph can carry Ag to lymphoid tissues after intraepidermal injection (69). The massive migration of DC in LN could play a key role in early MMTV infection to stimulate SAg-reactive T cells and therefore trigger the further T cell-B cell interaction required to complete the viral cycle (10).

Taken together, this study shows the infection of DC in addition to B cells early after MMTV injection. The infection of DC explains the normal priming of a SAG-induced immune response and the formation of a chronic SAg-driven immune response in the draining LN.

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References


