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Conditioning of Langerhans Cells Induced by a Primary CD8 T Cell Response to Self-Antigen In Vivo

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Using a previously described model of autoimmune skin disease, we addressed the question of how CD8 T cell responsiveness to self-Ag is regulated during chronic inflammation. In this model, CD8 T cells expand and induce tissue pathology directed at an epidermal self-Ag. However, we show here that this primary CD8 T cell response prevented subsequent expansion of a second CD8 T cell population with the same specificity. This lack of T cell accumulation was not due to Ag elimination, nor was it due to competition between the two T cell populations. However, skin-specific dendritic cells that present Ag in this model–Langerhans cells–underwent significant phenotypic changes associated with a compromised ability to stimulate naive T cells. Our study suggests that conditioning of dendritic cells may play a role in maintaining unresponsiveness to self-Ag during chronic inflammation. The Journal of Immunology, 2006, 176: 4658–4665.

Dendritic cells (DCs) are no longer viewed as simple initiators of the immune response but their role as immunoregulators is increasingly evident (1). Once regarded as pure signal receivers, T cells in turn have been shown to influence the activation and homeostasis of DCs as well (2). Therefore, the interaction between T cells and DCs can no longer be considered as a simple unidirectional relationship. CD8 T cell–DC interactions play a putative role not only in the initiation of the immune response but also in its modulation and termination (3). One mechanism by which T cells can execute their modulatory role is through the release of cytokines that condition DCs. Ruedl et al. (4) have shown that DC maturation occurred in vivo after viral infection in the absence of CD40 and CD4 T cell help and did not require viral infection of DCs but was mediated by peptide-specific CD8 T cells. In a related model, alloreactive, naive CD40L-negative CD8 T cells were able to respond to the MHC I-peptide complex with a rapid, DC-polarizing IFN-γ response (5). Complexity of T cell–DC interactions was further revealed by the finding that CTLA-4-Ig may be working by provoking DCs to catabolize tryptophan, thereby depriving T cells and contributing to their demise (6). Collectively, recent data indicate that the interaction between T cells and DCs is a complex bidirectional process that determines activation status and homeostasis of both its participants.

We have previously described a murine model where a neo-self Ag was expressed in keratinocytes (K14-OVAp) and resulted in a lethal CD8 T cell–dependent autoimmune disease when crossed to TCR transgenics (OT-I) that expressed a receptor specific for that Ag (7). Adoptive transfer of low numbers of naive OT-I T cells into K14-OVAp recipients resulted in extensive proliferation, development of effector and memory function, and induction of vitiligo. However, this skin disease did not progress and the animals survived long-term (8). It has been shown for self-Ag-specific CD4+ T cells that they pass through a significant effector stage on their way to an anergic state (9). This stage is characterized by production of effector cytokines, provision of help for CD8 T cells, and induction of in vivo pathology within organs that express cognate Ag. CD8 T cells in our system did not appear anergic, as they were able to respond to restimulation in vivo; yet, it is clear that some mechanism was controlling the autoimmune pathology in these mice. Therefore, we wanted to test whether modulation of DC Ag presentation by T cells was occurring in K14-transgenic mice, and whether it contributed to the control of autoimmune pathology.

Materials and Methods

Animals

C57BL/6 (B6), CB6F1, Thy1.1 congenic C57BL/6.PL, and CD45.1 congenic C57BL/6 mice were obtained from The Jackson Laboratory. OT-I mice express a transgenic receptor specific for the OVA 257–264 peptide (ova) in the context of the H-2Kb (10). Thy1.1-congenic OT-I-PL mice were obtained by backcrossing of OT-I mice to C57BL/6.PL mice. The transgenic strain expressing OT-I target Ag under the control of the human keratin 14 promoter was generated as described (7, 11). They are referred to as K14-OVAp. 2C mice (12) express an alloreceptor that also has reactivity to a synthetic peptide (SIVRYYGL), in the context of H-2Kb (13). K14-SIYp-transgenic constructs were generated using a multistep PCR procedure as previously described (11). Perforin KO/OT-I cells were provided by M. Dobrzanski (Trudeau Institute, Saranac Lake, NY). All mice were treated in accordance with federal guidelines approved by the University of Minnesota Institutional Animal Care and Use Committee.

T cell adoptive transfer

OT-I CD8 T cells were isolated from lymph nodes of OT-I, Thy1.1 or OT-I, Thy1.1/1.2 mice and purified by negative selection using magnetic cell sorting MACS (Miltenyi Biotech) as previously described (14). To purify CD4+ T cells, cell suspension was labeled with FITC-coupled Abs to B220, L-A, CD4, and CD44 (0.0125 μg of anti-B220 and anti-L-A per 1 × 106 cells; 0.004 μg of anti-CD4 per 1 × 106 cells) (all from BD Pharmingen). Following staining, cells were subject to depletion using anti-FITC MACS microbeads. Cell purity (>95%) was established by flow...
cytometry. A total of $5 \times 10^4$ purified Thy1.1 CD44lowCD8+ OT-I T cells were injected into the tail vein of recipient mice. At various times after the first adoptive transfer, a second population of purified Thy1.1+ Thy1.2+ CD44lowCD8+ OT-I T cells was injected into the tail vein of recipient mice. At various times after the second injection, single-cell suspensions from skin-draining lymph nodes or spleen were stained with PerCP-labeled anti-Thy1.1, allophycocyanin-labeled anti-Thy1.2, PE-labeled anti-CD8 (all from BD Pharmingen) to detect transferred cells. Data were acquired using a FACS-Calibur (BD Biosciences) and analyzed with FlowJo software (TreeStar). The number of OT-I T cells in lymph nodes and spleen were calculated by multiplying the percentage of Thy1.1+ Thy1.2+ CD8+ cells by the number of viable cells as determined by trypan blue dye exclusion. Three animals per experimental group were used. The number of OT-I cells was assessed for each mouse and an average number of OT-I cells from three animals was graphed. Alternatively, purified CD45.1+ CD44lowCD8+ OT-I T cells were used for the secondary transfer and they were detected using allophycocyanin-anti-CD45.1 and PE-labeled anti-CD8 (both from BD Pharmingen).

In some experiments, purified OT-I cells were labeled with CFSE (Molecular Probes) as previously described (15, 16).

**In vivo killing assay**

A single-cell suspension of C57BL/6 spleen cells was divided into two. One sample was pulsed with 0.2 μM OVA peptide (SIINFEKL) for 45 min at 37°C, washed, and labeled with a high concentration (1.5 nM) of CFSE (Molecular Probes). The other sample was incubated without peptide at 37°C for 45 min, washed, and labeled with a low concentration (0.05 mM) of CFSE. Equal numbers of CFSElow and CFSE+ cells were mixed together, and 2 $\times 10^7$ mixed cells were injected i.v. into recipients that had been previously transferred once or twice with congenic OT-I cells. After 3 h, mice were sacrificed and lymph nodes and spleen were analyzed by flow cytometry to detect CFSE-labeled cells.

The percent-specific lysis was determined by the following formula: ratio = (percentage CFSElow/percentage CFSEhigh); percent-specific lysis = (1 – (ratio unprimed/ratio primed)) × 100 (17).

**DC purification and in vitro stimulation**

DC from lymph node or spleen were prepared by digestion with collagenase D (Sigma-Aldrich) and EDTA as previously described (18). Cells were then labeled using MACS anti-CD11c MicroBeads (Miltenyi Biotec) and passed over a magnetized MACS selection column. For phenotypic characterization, DCs were stained with FITC-coupled Ab to CD205 (Serotec), PE-coupled Ab to CD11c, PerCP-coupled Ab to CD8α (both BD Pharmingen), biotin-labeled Ab to CD80, CD86, CD40 (all eBioscience), and purified anti-E-cadherin (Sigma-Aldrich). Some DCs were also stained for E-cadherin expression using an Ab (6C2) provided by Dr. D. Saelens (Laboratory for Immunological Research, Schering-Plough) as previously described (19). Goat-anti-rat IgG (BD Pharmingen) was used as secondary Ab for E-cadherin and Langerin staining.

**Intracellular cytokine staining following in vitro rechallenge**

First, $5 \times 10^5$ CD44lowCD8+ OT-I-Thy1.1 cells, and 6 days later $5 \times 10^5$ CD44lowCD8+ OT-I-Thy1.1Thy1.2 cells, were transferred i.v. into K14-OVAp or C57Bl/6 mice. Six days after the second transfer, lymph nodes and spleen were collected and cells were incubated in RPMI 1640+ 10% FCS with 2 μM OVA. After 2 h of incubation, 1 μl/ml GolgiPlug (BD Pharmingen) was added and cells were incubated for additional 4 h at 37°C. Cells were washed and stained with anti-CD8, anti-Thy1.1, and anti-Thy1.2 Abs to mark the second OT-I population. Cells were fixed in Cytofix/Cytoperm solution (BD Pharmingen) for 20 min at 4°C before staining with PE-conjugated Ab to IFN-γ (eBioscience) for 30 min at 4°C. Cells were washed in Perm/Wash solution (BD Pharmingen) and resuspended in FACs buffer. Data were collected on a FACS-Calibur (BD Biosciences) and analyzed with FlowJo software (TreeStar).

**In vivo depletion of OT-I-Thy1.1+ cells**

On days 8, 9, and 10 after the adoptive transfer of OT-I cells, recipient mice were injected i.p. with 400 mg of anti-Thy1.1/mouse/day (1A14, ascites from Maine Biotechnology Services). The efficiency of depletion was determined by flow cytometry of lymph node cells from control and anti-Thy1.1-depleted mice stained with anti-CD8 and anti-Thy1.1 (clone OX-7; BD Pharmingen).

**Bone marrow chimeras**

K14-OVAp (C57BL/6 CD45.2) mice were given a lethal dose of irradiation (1000 rad) and subsequently injected with $10^7$ bone marrow cells from congenic C57BL/6.CD45.1 or C57BL/6.bm1 mice. Chimerism of lymph node DC and epidermal Langerhans cells (LC) was evaluated using an Ab to CD45 (for the B6 224 B6.K14 chimeras) or a combination of Abs to murine Kb–5F1 and Y3. The Y3 Ab recognizes both Kb and Kb1m, whereas 5F1 recognizes Kb, but not Kb1m.

**Results**

A primary response of OT-I cells in K14-OVAp mice prevents subsequent expansion of a second OT-I population.

We previously used an adoptive transfer approach to test the response of CD8 T cells to a neo-self peptide Ag synthesized in keratinocytes. Naive OT-I CD8 T cells transferred into K14-OVAp recipients proliferated extensively, migrated to tissues, developed effector function, and were capable of making a recall response (8). We showed that transfer of high numbers of naive CD8 T cells into peptide-transgenic mice caused a lethal autoimmune reaction, whereas 5F1 recognizes Kb, but not Kb1m.
DC8 OT-I.Thy1.1/1.2 cells from lymph nodes and spleen is shown. Graphs are representative of several independent experiments. Temporal setup. K14-OVAp or control B6 mice were injected with 5 x 10⁴ naive CD8 OT-I.Thy1.1 cells or left without transfer and 14 days later, 5 x 10⁴ naive CD8 OT-I.Thy1.1/1.2 cells were transferred into the same mice. Six days after the second transfer, proliferation of the secondary population was assessed as expansion of Thy1.1⁺/1.2⁺ cells by flow cytometry. A. The kinetics of expansion of OT-I.Thy1.1/1.2 cells transferred directly in K14-OVAp recipients (primary), or transferred into recipients that received OT-I.Thy1.1 cells 14 days earlier (secondary), or transferred into non-Ag-bearing controls 10⁴ naive CD8 OT-I.Thy1.1/1.2 cells were transferred into the same mice. Six days after the second transfer, proliferation of the secondary population was assessed as expansion of Thy1.1⁺/1.2⁺ cells by flow cytometry. B. The experiment was performed as in A and cells were restimulated in vitro with OVAp and stained intracellularly for IFN-γ. Mean fluorescence intensity (MFI) of IFN-γ fluorescence in lymph node Thy1.1⁺/1.2⁺ cells is shown. C. The total numbers of CD8 OT-I.Thy1.1/1.2 cells from lymph nodes at the peak day of expansion (day 6) are shown. D. The experiment was performed as in A and cells were restimulated in vitro with OVAp and stained intracellularly for IFN-γ. Mean fluorescence intensity (MFI) of IFN-γ fluorescence in lymph node Thy1.1⁺/1.2⁺ cells is shown. E. The experiment was performed as in A. Time between the primary and the secondary transfer was extended to 50 days. Numbers of CD8 OT-I.Thy1.1/1.2 cells from lymph nodes are shown. F. Different numbers of naive CD8 OT-I.Thy1.1 were transferred into K14-OVAp recipients and 14 days later, 5 x 10⁴ naive CD8 OT-I.Thy1.1/1.2 cells were transferred into the same mice. Total number of CD8 OT-I.Thy1.1/1.2 cells from lymph nodes and spleen is shown. Graphs are representative of several independent experiments.

Strikingly, only 10⁴ OT-I cells were needed to induce inhibition of the second T cell population and as few as 100 cells reduced the expansion by >10-fold (Fig. 1F).

Taken together, these results imply that the initial Ag-specific response of OT-I cells in K14-OVAp mice induces a long-lasting state of unresponsiveness in these mice. We were interested in understanding the mechanism involved, and whether it was Ag specific.

The lack of T cell accumulation was not due to Ag elimination

As shown previously (8), OT-I cells gained cytolytic effector function in K14-OVAp recipients. Several published findings indicate that Ag-loaded DCs are targets for activated CTL in vivo (20–22). Thus, it was possible that the primary population induces tolerance by cytolytic elimination of APC presenting the OVA peptide, and consequently limited the autoimmune response. To test whether elimination of APC was responsible for impaired proliferation of the second OT-I population, we measured up-regulation of a classic T cell activation marker, CD69, on the second OT-I cells in K14-OVAp mice. Several recent reports described acquisition of molecules by T cells from APCs. These have included the transfer of MHC class I (23, 24), costimulatory (25, 26), and MHC class II molecules (27). Also, high-affinity T cells were shown to down-modulate peptide-MHC complexes from APCs and deprive the lower affinity T cells of adequate levels of cognate ligand (28). This view has been recently challenged (29, 30); however, down-modulation or transfer of peptide-MHC complexes from APC to T cell represents a potential mechanism of immunoregulation that may play a role in tolerance induction to self-Ag. Therefore, we wanted to address whether continuous presence of the initial OT-I T cells was needed for the control and/or inhibition of the second population. OT-I cells were transferred into two groups of K14-OVAp mice. One group received naive OT-I cells alone, and the other also received naive OT-I cells followed by naive OT-I cells that had been preactivated with OVA. The latter group showed no difference in the control of the second OT-I population compared to the group that received naive OT-I cells alone. These results suggest that the initial OT-I cell population does not need to be present to inhibit the proliferation of the second OT-I population. Instead, the initial OT-I cell population may have induced a state of unresponsiveness that is maintained even after the initial presence of OT-I cells is lost. This conclusion is further supported by the finding that the primary responders were perforin deficient or not. It was found that the lack of T cell accumulation was not due to Ag competition but rather to a mechanism that was specific to the initial OT-I cell population. The data suggest that the initial OT-I cell population plays a role in preventing the accumulation of the second OT-I cell population.

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group was left intact while the second group was treated with anti-Thy1.1-depleting Ab (1A14) on days 8, 9, and 10 (Fig. 3A). The efficiency of OT-I cell depletion was confirmed by flow cytometry (Fig. 3B). Fourteen days later, both groups were transferred with the second population of OT-I cells. To prevent potential depletion of the second OT-I population by the residual anti-Thy1.1 Ab, we used OT-I cells with a different congenic marker (CD45.1). There was no difference in the OT-I cell accumulation between the group that underwent depletion and the one that did not (Fig. 3C), suggesting that the continued presence of the original responding T cells is not needed for the tolerogenic properties observed in this system. Interestingly, the same phenomenon was observed when the time period between depletion and the second adoptive transfer was extended to 50 days (Fig. 3D), implying that tolerance is long-lasting in the absence of the first OT-I population.

To further confirm the effectiveness of OT-I depletion with anti-Thy1.1 Ab as well as the lack of effector function of the second OT-I T cell population, we performed an in vivo killing assay. On day 6 after the second adoptive transfer, both recipient groups received unpulsed or OVA-pulsed B6 spleen cells. The two populations were differentially CFSE labeled and the killing of OVA-pulsed cells was assessed as a disappearance of the CFSE<sup>high</sup> population by flow cytometry (31). Although OVA-pulsed targets remained intact in B6 hosts, 98% killing efficiency was observed in K14-OVAp control hosts (Fig. 3E). No killing was observed in K14-OVAp hosts where the first OT-I population was depleted, suggesting that the second population of T cells was not able to develop cytolytic effector function. This is consistent with the lack of IFN-γ production by the second population (Fig. 1D). Interestingly, the residual killing capacity observed in K14-OVAp recipients where the first population of OT-I cells was left intact was eliminated with the anti-Thy1.1 treatment. That the initial population could be effective killers but the secondary population could not, even in the same animal, suggests the importance of priming conditions in determining effector function. Thus, in stark contrast to the response of the primary CD8 T cell population, the Ag-presenting environment in which the second population gets activated leads to an abortive activation response, where T cells divide, but do not expand or acquire effector function.

**Endnote**

Abortive migration and transient phenotypic changes of LC

Aborting migration of CD8 T cells, such as we observe here, was also seen in models where pancreatic self-Ags were presented by lymph node DC in the steady state. This and other data have led to the idea that DC maintain immunological tolerance under steady-state conditions (32). LC may represent an exception from this rule because the majority of LC in the lymph nodes bear mature phenotype (33). Whether this is due to a continuous basal level of skin irritation (due to scratching) or represents true steady state remains to be elucidated. Also, in the situation where self-Ag was expressed in the skin, LC induced activation of self-Ag-specific T cells that subsequently led to autoimmune disease (8, 34). In contrast, it has been shown during chronic human skin disease that inflammatory stimuli in the skin increased the migration of LC to the lymph node but these cells had an immature phenotype (35). Consequently, LC conditioned under inflammatory stimuli might down-regulate immune responses in reaction to skin inflammation.

To test whether phenotypic changes of LC were induced after adoptive transfer of OT-I cells into K14-OVAp mice, we isolated DC from skin-draining lymph nodes of control B6 mice or K14-OVAp mice after adoptive transfer of OT-I cells. We confirmed that the LC phenotype in B6 and K14-OVAp mice without adoptive transfer is identical (data not shown), which allowed us to use B6 mice as proper controls in our phenotypic analysis. Under steady-state conditions, skin migrant DC (DEC205<sup>high</sup>CD8<sup>low</sup>) represented ~15–25% of DCs in the lymph nodes (Fig. 4A). In K14-OVAp mice after adoptive transfer of OT-I cells, these cells became activated as assessed by reduction of DEC205 expression (36) and their percentage as well as numbers in the lymph node increased at least 2-fold (Fig. 4A and data not shown). To confirm that DEC205<sup>high</sup>CD8<sup>low</sup> were indeed LC, we stained the cells with Ab to a LC-specific marker, Langerin (CD207). As shown in Fig. 4B, DEC205<sup>high</sup>CD8<sup>low</sup> cells in B6 mice and DEC205<sup>high</sup>CD8<sup>low</sup> cells in K14-OVAp mice after adoptive transfer were Langerin positive. Expression of E-cadherin and CD83 on LC also remained unchanged upon adoptive transfer (Fig. 4C). In contrast, CD86, CD40, and MHC class II expression decreased. This phenotypic change was particularly interesting in the context of a previous finding that in neonatal mice, low expression of costimulatory molecules on LC contributed to tolerance induction to skin Ags (37). Furthermore, expression of two recently described ligands for programmed cell death (PD)-1, PD-L1 (B7-H1) and PD-L2 (B7-DC),
were increased after adoptive transfer of OT-I cells into K14-
OVAp mice implying a potential role of LC in negative regulation
of T cell responses.

Bone marrow chimera analysis supports a role for LC

Although phenotypic changes were observed in migrating LC on
day 6 after the autoimmune response, it is possible that other APC
were responsible for the different functional response of the sec-
todary population. In particular, we considered the scenario when cyto-
lytic CD8 T cells in the epidermis could cause the release of
increased amounts of Ag, triggering their presentation by other APC,
such as CD8

DC in the skin-draining lymph node. To test this, we
analyzed the response in bone marrow chimeras where conven-
tional lymphoid and myeloid DC could not present the Ag. OT-I T
cells do not recognize the OVAp Ag when presented by Kbml,
therefore in bm1

B6.K14 chimeras, the predominant DC capable
of presenting Ag to naive T cells in the skin-draining nodes is the
epidermal LC, because it is radioresistant and other DC are not

(38). In such chimeras, we found that responsiveness of the sec-
todary T cell population was still suppressed (Fig. 5), suggesting
that LC are the relevant APC both in initiating the autoimmune
response (8), and in the resultant down-regulation.

Effect on other specificities is consistent with DC conditioning,
but only at early time points

The phenotypic changes of LC shown in Fig. 4C represented a transient phenomenon. When the phenotype was examined on day
30 after adoptive transfer, no major difference was observed be-
tween LC from K14-OVAp and B6 mice (Fig. 4D). The only sur-
face marker that retained some of the transient characteristics was
CD40. However, this difference disappeared by day 90 (data not
shown); despite the fact that the second OT-I population remained
unresponsive at later time points, days 50 and 90 (Fig. 1E and data
not shown). Thus, the transient nature of the DC phenotypic
changes may explain the initial impairment of OT-I responsiveness

FIGURE 4. Enhanced migration and phenotypic changes of LC early after adoptive transfer of OT-I cells into K14-
OVAp mice. A. A total of 5 \times 10^6 naive CD8 OT-I.Thy1.1 cells were transferred into two groups of K14-OVAp mice and were
depleted in one group of K14-OVAp mice by anti-Thy1.1-depleting Ab on days 8, 9, and 10. Four days after depletion, naive CD8 OT-LC-CD45.1 cells were transferred into control
animals and both groups of previously transferred mice. On
day 6 after the secondary adoptive transfer, proliferation of
CD51+ cells was assessed using flow cytometry. B. A total of
5 \times 10^6 naive CD8 OT-I.Thy1.1 cells were transferred into
two groups of K14-OVAp mice and were depleted in one
group by anti-Thy1.1-depleting Ab on days 8, 9, and 10. Four
days after depletion, the presence of remaining Thy1.1+ cells
was assessed by flow cytometry. C. The experiment was per-
formed as in A. OT-I CD8 T cell expansion on day 6 after the
second adoptive transfer was assessed by flow cytometry. D.
The experiment was performed as in C except that the time
between Ab depletion treatment and the secondary transfer
was extended to 50 days. E. The experiment was conducted as
described in A except that on day 11 (one after depletion)
naive CD8 OT-LC-CD45.1 cells were transferred. Six days later,
the animals received target cells labeled with CFSE. The
OVAp-pulsed target cells were labeled with a high level of
CFSE and the control target cells were labeled with a lower
level of CFSE. Percent-specific lysis was calculated as de-
scribed in Materials and Methods. All graphs are representa-
tive of several experiments.
in K14-OVAp mice, but other factors must play a role in the long-lasting unresponsiveness of the second OT-I T cell population.

If DC conditioning was responsible for the reduced responsiveness, then one would predict that the response to any other Ag presented by LC would also be affected. To test this, we took advantage of mice expressing a different peptide Ag (the 2C target Ag) under the control of the K14 promoter (K14-SIYp). Those mice were bred to K14-OVAp mice to gain offspring with expression of two Ags under the same promoter (K14-OVAp/SIYp). Naive OT-I cells were transferred into K14-OVAp/SIYp double-transgenic mice and 6 days later 2C.Rag2-/- cells were transferred into the same recipients and control mice. Six days after the secondary transfer, lymph nodes and spleen were harvested and proliferation of 2C cells was assessed by flow cytometry. As shown in Fig. 6A, a partial reduction of 2C proliferation was observed in this experiment (50-fold less expansion). The secondary transfer in this experiment was performed within the timeframe of DC phenotypic changes and is consistent with a nonspecific “DC conditioning” effect.

To test whether this effect was transient or long-lasting, we extended the time between the first and the second adoptive transfer to 21 days. As shown in Fig. 6B, no inhibition of 2C proliferation was observed at this later time point, suggesting that the limited responsiveness in K14-OVAp mice is ultimately Ag specific. Overall, our data suggests that an autoimmune CD8 T cell response to an epidermal Ag causes a transient reduction in the ability of skin-migrating DC to support T cell activation, and this correlates with altered expression of costimulatory molecules. Ultimately however, additional mechanisms, yet to be identified, control T cell responsiveness to the original autoantigen.

Discussion

The K14-OVAp model provides a powerful tool to study CD8 T cell peripheral tolerance mechanisms. In these mice, central tolerance seems to operate relatively normally because the thymic profile of OT-I × K14-OVAp mice shows a profound reduction of mature CD8SP (39) and because immunization of K14-OVAp mice with vaccinia-OVA does not result in the expansion of Kb/ OVAp tetramer-binding CD8 T cells (data not shown). However, peripheral tolerance does seem to be defective because naive OT-I T cells undergo extensive proliferation, gain effector function, and cause autoimmune pathology (8). However, at low transfer numbers, animals survive and disease does not progress. In this study, we showed that the DC subset that presents self-Ag to CD8 T cells in the lymph nodes–LC (8)—changed expression of surface molecules and reversed to a semimature phenotype during the course of the autoimmune response. This change was associated with a dramatically different response of a second population of naïve T cells specific for the same Ag. The response of naïve T cells specific to an unrelated epidermal Ag (SIYp) was affected as well (Fig. 6A), at least transiently. One could hypothesize that conditioning of DCs becomes important when the immune response to self-Ag cannot be terminated by cytolytic elimination of Ag-bearing DCs, such as occurs during the response to foreign Ag (40). Therefore, the immune system has to substitute with alternative control mechanisms. Indeed, Geissmann et al. (35) have shown in human dermatopathic lymphadenitis that an expanded population of LC in the draining lymph nodes associated with affected skin had an immature phenotype expressing lower levels of CD86. These findings indicated that LC migration and maturation can be independently regulated events and suggested that LC might regulate immune responses during chronic inflammation. Therefore, our experimental results provide further evidence that LC migration and maturation can be uncoupled and make the K14-OVAp mouse a potentially valuable animal model for the study of diseases that involve chronic skin inflammation.

However, LC conditioning cannot be regarded as a sole mechanism responsible for the long-term changes in responsiveness of CD8 T cells to skin autoantigens. From our results, it is clear that a single self-Ag-specific CD8 T cell response initiates changes that dramatically alter subsequent CD8 T cell responses to that same Ag. Indeed, the anti-Thy1.1 elimination experiments in Fig. 3 reveal that even a transient CD8 T cell response to self sets up this situation. Recent evidence suggests that DC induce Ag-specific regulatory cells under certain conditions (32, 41). We have tried to identify a potential regulatory cell among lymphocytes in our system. Surprisingly, neither CD8−, CD4−, nor double-negative T cells harvested from K14-OVAp mice after the first adoptive transfer were able to transfer tolerance to a new K14-OVAp recipient...


