Presentation of the Self Antigen Myelin Basic Protein by Dendritic Cells Leads to Experimental Autoimmune Encephalomyelitis

Bonnie N. Dittel, Irene Visintin, Raina M. Merchant and Charles A. Janeway, Jr.

http://www.jimmunol.org/content/163/1/32

**References**  This article cites 42 articles, 26 of which you can access for free at:
http://www.jimmunol.org/content/163/1/32.full#ref-list-1

**Why The JI?** Submit online.

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

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

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

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Presentation of the Self Antigen Myelin Basic Protein by Dendritic Cells Leads to Experimental Autoimmune Encephalomyelitis

Bonnie N. Dittel, Irene Visintin, Raina M. Merchant, and Charles A. Janeway, Jr.

Bone marrow (BM)-derived dendritic cells (DC) are potent stimulators of naive CD4\(^+\) T cell activation. Because DC are efficient at Ag processing and could potentially present self Ags, we investigated the role of DC in the presentation of an encephalitogenic peptide from myelin basic protein (Ac\(_{1-11}\)) in the induction of experimental autoimmune encephalomyelitis (EAE). To determine if DC could prime for EAE, we transferred DC pulsed with Ac\(_{1-11}\) or with medium alone into irradiated mice in combination with CD4\(^+\) T cells isolated from a mouse transgenic for a TCR specific for Ac\(_{1-11}\) + I-A\(^b\). Mice transferred with Ac\(_{1-11}\)-pulsed DC developed EAE 7–10 days later, whereas mice receiving medium-pulsed DC did not. By day 15, all mice given peptide-loaded DC had signs of tail and hind limb paralysis, and by day 20 infiltration of Ac\(_{1-11}\)-specific CD4\(^+\) T cells was detected in the brain parenchyma. We also demonstrated interactions between Ac\(_{1-11}\)-pulsed DC and Ac\(_{1-11}\)-specific T cells in the lymph nodes 24 h following adoptive transfer of both cell populations. These data show that DC can efficiently present the self Ag myelin basic protein Ac\(_{1-11}\) to Ag-specific T cells in the periphery of mice to induce EAE.


Dendritic cells (DC)\(^1\) are specialized APC that possess the capacity to activate naive T cells (1, 2). DC residing in the T cell areas of lymph nodes are of bone marrow (BM) origin (3) and have been shown to migrate from the peripheral blood into the spleen and from afferent lymphatics into the lymph nodes following activation, presumably after encountering Ag (4, 5). Once established in the lymph node, DC characteristically express MHC class II molecules and many of the adhesion molecules (ICAM-1, LFA-1, and LFA-2) involved in interaction with naive T cells and the costimulatory molecules (B7-1, B7-2, heat stable Ag, and CD40) needed for naive T cell priming (1, 6). In particular, MHC class II and B7-2 are constitutively expressed at very high levels (1, 6, 7). The morphology of DC facilitates interaction with T cells by means of thin cytoplasmic sheets, also referred to as veils, extending as processes into the surrounding lymph node (1). Visualization of interactions between DC and T cells in the T cell-rich areas of lymph nodes have been demonstrated for T cells in response to a specific peptide Ag (8).

It has been hypothesized that T cell-mediated autoimmune diseases are the result of inappropriate Ag presentation of either a self-Ag or an Ag with the capacity to mimic a self-Ag in the peripheral lymphoid tissues. An activated or memory T cell specific for a self peptide then has the potential to meet the self Ag where it is naturally expressed. In the case of experimental autoimmune encephalomyelitis (EAE), autoreactive T cells would re-encounter their specific self-Ag in the CNS. Immunization of mice with several different protein components of myelin, including myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein, are capable of eliciting an immune response resulting in the quintessential symptoms of EAE: ascending paralysis involving the tail and then the limbs (9, 10). EAE is a demyelinating disease in rodents and is used as a model of the human disease multiple sclerosis (9–11). EAE is characterized by focal areas of inflammation and demyelination throughout the CNS and can be actively induced in genetically susceptible animals by the injection of myelin Ags in CFA or passively by the adoptive transfer of activated CD4 T cells producing type 1 cytokines specific for myelin Ags, provided they express the adhesion molecule VLA-4 (9, 10, 12). In I-A\(^b\) mice, the primary immunogenic peptide of MBP is the acetylated N-terminal peptide from residues 1 to 11 (Ac\(_{1-11}\)) (9).

In this study, we show that DC capable of processing and presenting the self Ag MBP migrate to the lymph nodes via the afferent lymphatics, and interact with naive Ac\(_{1-11}\)-specific T cells leading to the induction of EAE. In addition, Ac\(_{1-11}\)-specific T cells were observed in the brains and spinal cords of mice 20 days following this unique means of EAE induction. These data demonstrate that presentation of a self Ag by DC in the periphery results in the activation of MBP-specific T cells and can lead to an overt autoimmune disease.

Materials and Methods

Mice

B10.PL mice (I-A\(^b\)) were purchased from The Jackson Laboratory (Bar Harbor, ME). MBP-TCR transgenic (tg) mice were generated as previously described (13) and reared in our colony at Yale University. All mice used were between 5 and 10 wk of age.

Peptides and Abs

The MBP Ac\(_{1-11}\) (Ac-ASQKRPSQRSK) and the MBP\(_{121-140}\) (GFGYG GRASDYGSAHKGFKG) peptide were synthesized and HPLC purified by...
Cells

DC were isolated as previously described (14). Briefly, BM was flushed from the femurs and tibias of B10.PL mice. BM cells were depleted of B cells, T cells, and I-A\(^+\) cells using Abs and complement. The mAb used were GK1.5, HO-2.2, Y19, RA3-3A1/6.1, and Y3JP. The remaining mono-nuclear cells were plated in 12-well plates at 0.5 \(\times 10^6\) cells/ml in RPMI 1640 medium supplemented with 5% FCS, 1 mM L-glutamine, 20 \(\mu\)g/ml gentamicin, 50 \(\mu\)M 2 ME, and 1% culture supernatant from 358L cells transfected with a GM-CSF construct (kindly provided by Fritz Melchers, Basel Institute of Immunology, Basel, Switzerland). After 2 days, the non-adherent cells were gently removed and discarded, and fresh medium was added every 2 days to the remaining adherent cells. On day 6, the non-adherent and loosely adherent cells were removed and replated into 100-mm culture tissues in the above medium. Following overnight incubation, the nonadherent cells were collected and pulsed with medium alone or Ac\(_{1–11}\) (100 \(\mu\)g/ml) for up to 6 h in 100-mm culture dishes and washed twice in PBS before transfer into mice. Purity of the DC was \(>60\%\) as determined by flow cytometry examining expression of B-7 and high levels of MHC class II (Y3JP). MBP-TCR CD4 T cells were isolated from the spleen of MBP-TCR tg mice. Briefly, spleens were minced and RBC were removed following depletion of cells expressing CD8, B220, and I-A\(^+\) using mAb MBP-TCR tg mice. Briefly, spleens were minced and RBC were removed following depletion of cells expressing CD8, B220, and I-A\(^+\) using mAb and complement. The mAb used were HO-2.2, RA3-3A1/6.1, and Y3JP. Purity of the T cells was assessed using mAb specific for mouse CD4, CD8, B220, \(\beta\)2\(\beta\) TCR, V\(\beta\)8.1.2, and the MBP-TCR clonotypic mAb 19G. The MBP-TCR 31 clone was obtained from the spleen of a MBP tg mouse we prepared (13) that expresses the TCR specific for Ac\(_{1–11}\) and restricted to I-A\(^d\). The MBP-TCR 31 clone was maintained by restimulation in Click’s Eagle Hank’s amino acid (EHAA) medium containing 10% FCS, 2 U/ml IL-2, and 5 \(\mu\)g/ml Ac\(_{1–11}\) in the presence of inactivated spleen cells from B10.PL mice (I-A\(^d\)) every 3–6 wk and allowed to rest at least 10 days before use. The MBP-TCR 31 clone is CD4\(^+\) and expresses the MBP-TCR as assayed by staining with mAb 19G.

T cell activation assay

MBP-TCR 31 cloned cells (\(1 \times 10^5\)) or splenic CD4\(^+\) MBP-TCR CD4 T cells (\(1 \times 10^4\)) were incubated with 1 \(\times 10^4\) BM-derived DC isolated as described above in the presence or absence of 0.1–10 dilutions of the peptides Ac\(_{1–11}\) or MBP\(_{121–140}\) from 0.001 to 1 \(\mu\)g/ml in Click’s EHAA medium containing 5% FCS. In addition, 1 \(\times 10^5\) cloned T cells were incubated with increasing numbers of DC that had been pulsed with medium alone, 100 \(\mu\)g Ac\(_{1–11}\), or 100 \(\mu\)g/ml whole mouse MBP (mMBP). CD4\(^+\) T cells (2 \(\times 10^5\)), isolated from the spleen of a mouse 30 days posttransfer of Ac\(_{1–11}\)-pulsed DC and MBP-TCR CD4 T cells (as described below), were cocultured with 2 \(\times 10^5\) inactivated H-2\(^b\) splenocytes in the presence of increasing concentrations of Ac\(_{1–11}\). Proliferation was detected at 72 h by the addition of 1 \(\mu\)Ci [\(^{3}H\)]Tdr to each well for the last 15–18 h of culture. Individual data points were set up in triplicate.

Cytokine secretion

IL-2 production from splenic CD4\(^+\) MBP-TCR CD4 T cells was detected using CTL-2 responder cells. Culture supernatants were collected 24 h following coculture of 1 \(\times 10^6\) MBP-TCR CD4 T cells and 1 \(\times 10^5\) inactivated H-2\(^b\) splenocytes in the presence of increasing concentrations of Ac\(_{1–11}\). Prior to the addition of 5000 CTL-2 cells, the culture supernatants were frozen at \(-70^\circ\)C to kill any viable cells. Following culture for 18 h, the cells were pulsed with 1 \(\mu\)Ci [\(^{3}H\)]Tdr and harvested after 4 h. IFN-\(\gamma\) was detected from culture supernatants collected 24 h following coculture of 2 \(\times 10^5\) CD4\(^+\) splenic T cells, isolated from a mouse injected 30 days prior with Ac\(_{1–11}\)-pulsed DC and splenic MBP-TCR CD4 T cells, and 2 \(\times 10^5\) inactivated H-2\(^b\) splenocytes in the presence of increasing concentrations of Ac\(_{1–11}\). IFN-\(\gamma\) was detected by ELISA as previously described (15).

EAE induction

Irradiated (600 rads) female B10.PL mice were s.c. injected in the footpads (0.5 \(\times 10^6\) cells) and in each internal flank (1 \(\times 10^6\) cells) with BM-derived DC in PBS that had been pulsed with medium alone or Ac\(_{1–11}\). Twenty-four hours later, 10 \(\times 10^6\) MBP-TCR CD4 splenic T cells in PBS, isolated as described above, were injected i.v. into each animal. Individual animals were assessed daily starting at day 6 for symptoms of EAE and scored using a scale from 1 to 5 as follows: 0, no disease; 1, limp tail and/or wobbly walk; 2, hind limb paraparesis; 3, hind limb paralysis; 4, hind and fore limb paralysis; 5, death.

Labeling and in vivo detection of T cells and DC

DC and MBP-TCR tg T cells isolated as described above were labeled with the fluorescent dyes 3.3’,3’dioctadecylxocarboxyanine perchlorate (DHO) (6 mg/nl/ml and 1.1–diododecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI, 2.5 mg/ml), Molecular Probes, Eugene, OR), respectively, for 30 min at 37\(^\circ\)C after which they were washed three times in PBS. DC (0.5 \(\times 10^6\) pulsed with medium alone or with 100 \(\mu\)g/ml Ac\(_{1–11}\) were injected s.c. into each footpad followed by i.v. injection of 10 \(\times 10^6\) MBP-TCR tg CD4 T cells. After 24 h, the popliteal lymph nodes were harvested and fixed in paraformaldehyde-lysine-periodate overnight. The lymph nodes were sucrose infused prior to embedding in Tissue Tek OCT (Miles, Elkhart, IL) and frozen in isopentane. Frozen sections (7 \(\mu\)m thick) were generated and visualized on a fluorescent microscope.

Immunohistopathological analysis

Brains and spinal cords were removed from perfused mice and fixed in paraformaldehyde-lysine-periodate. After 24 h, the tissues were sucrose infused, embedded in Tissue Tek OCT (Miles), and frozen in isopentane. For immunohistochemistry, frozen sections (7 \(\mu\)m thick) were stained with biotinylated anti-CyD4, biotinylated anti-V\(\beta\)8.1,8.2, or biotinylated 19G. The color was developed using HistoMark Red Phosphatase System (Kiregaard & Perry Laboratories, Gaithersburg, MD) and counterstained with hematoxylin.

Results

BM-derived DC present a MBP self peptide to a MBP-specific T cell clone

To examine the presentation of a MBP self peptide by DC, we derived DC from the BM of the B10.PL mice (H-2\(^b\)), a mouse strain that is genetically susceptible to the CNS autoimmune disease EAE. To detect Ag presentation by the DC, we used a CD4\(^+\) T cell clone (MBP-TCR 31) isolated from a mouse tg for the rearranged TCR \(\alpha\)- and \(\beta\)-chains of a cloned T cell line called clone 19 that is restricted to I-A\(^d\) and specific for Ac\(_{1–11}\) (12). As shown in Fig. 1A, proliferation of the MBP-TCR 31 clone occurred following presentation of the Ac\(_{1–11}\) peptide by BM-derived DC in a dose-dependent manner. The specificity of the MBP-TCR 31 clone to Ac\(_{1–11}\) is demonstrated by the lack of proliferation to the MBP peptide composed of residues 121–140, which binds to I-A\(^a\) and is encephalitogenic in (B10.PL \(\times\) SJL)F1 mice (B. N. Dittel and C. A. Janeway, unpublished observations). Because the goal of this study was to use Ac\(_{1–11}\)-pulsed DC in vivo to generate an immune response culminating in EAE, we examined the proliferative response of the MBP-TCR 31 clone to Ac\(_{1–11}\)-pulsed DC. As shown in Fig. 1B, proliferation of the MBP-TCR 31 clone was enhanced with the addition of increasing numbers of Ac\(_{1–11}\)-pulsed DC. In contrast, no proliferation was observed when the DC were pulsed with medium alone. Because Ag uptake and processing have been shown to change with DC maturation (17), we tested whether the DC used in this study were capable of protein Ag uptake leading to processing and presentation of the Ac\(_{1–11}\) peptide. This is demonstrated in Fig. 1C by the proliferative response of the MBP-TCR 31 to DC pulsed with whole mMBP.

Direct interaction between Ac\(_{1–11}\)-pulsed DC and MBP-TCR tg T cells is observed in the draining lymph node

DC have been shown to form clusters with Ag-specific T cells in vitro (18) and in vivo (8). Because this clustering is believed to
medium alone were transferred with MBP-TCR CD4 T cells, few cell-cell interactions and little T cell-DC clustering was observed as shown by high power (Fig. 2C) and by low power (Fig. 2A) magnifications. These data show that the interaction between DC presenting the Ac1–11 peptide and the MBP-TCR CD4 T cells are specific for self peptide.

**Ac1–11-pulsed DC result in Th1 priming of naive MBP-TCR T cells**

We chose to use CD4 T cells isolated from MBP-TCR tg mice in our experiment in Fig. 2 because DC have been shown preferentially to interact with and activate naive T cells. CD4+ T cells isolated from the spleen of a MBP-TCR tg mouse are 95% Vβ8.2+ (data not shown), the β-chain expressed by the MBP TCR. We found that the Vβ8.2+ T cells, >98% were of the naive phenotype as determined by the expression of low levels of CD44 and high levels of CD45RB. Thus, only 1.2–1.5% of the Vβ8.2+ T cells expressed the memory phenotype, CD44high and CD45RBlow (data not shown). The specificity of the MBP-TCR CD4 T cells is shown in Fig. 3A by the dose-dependent proliferation and IL-2 production following stimulation with Ac1–11. Because we detected a small population of memory cells that are known to produce abundant quantities of either Th1 or Th2 cytokines upon stimulation, we examined the MBP-TCR CD4 T cells for the production of IL-4 and IFN-γ (Fig. 3A). We did not detect either IL-4 or IFN-γ by ELISA (data not shown). The production of IL-2 in the absence of IL-4 and IFN-γ further suggests a naive phenotype of the tg T cells. To examine whether we could prime for a Th1 or Th2 response using DC, we isolated CD4+ splenocytes from mice 30 days following transfer of Ac1–11-pulsed DC and MBP-TCR CD4 T cells and tested for proliferation and cytokine production. The successful priming of MBP-TCR T cells is shown in Fig. 3B by the dose-dependent proliferation and IFN-γ production in response to stimulation with Ac1–11. No IL-4 production was detected (data not shown), demonstrating priming of a Th1, but not Th2, response.

**Interactions between Ac1–11-pulsed DC and MBP-TCR CD4 T cells in vivo results in EAE**

Because we were able to demonstrate an Ag-specific interaction between DC and MBP-TCR CD4 T cells in vivo in lymph nodes leading to a Th1 response, we asked whether this interaction could lead to the induction of EAE. As shown in Fig. 4A, symptoms of EAE occurred as early as day 8 following adoptive transfer of naive MBP-TCR CD4 T cells and Ac1–11-pulsed DC. As a comparison, animals that were transferred with MBP-TCR CD4 T cells and medium-pulsed DC exhibited no signs of EAE (Fig. 4A). All animals that received peptide-loaded DC exhibited signs of EAE by day 11, and peak disease was reached on day 20. The animals were sacrificed on day 20 at the peak of disease for the analysis of CNS cell infiltration (Fig. 5). In an extended experiment in which the animals continued being examined for EAE after the peak day of disease, the mice were able to resolve most symptoms of disease and recover (Fig. 4B). These data show that DC can present the Ac1–11 self peptide to MBP-TCR T cells resulting in autoimmunity. The induction of EAE is dependent upon the transfer of live DC, as i.p. injection of Ac1–11 (100 µg) or transfer of paraformaldehyde-fixed DC pulsed with Ac1–11 in combination with transferred MBP-TCR T cells did not result in EAE. In the experiments shown in Fig. 4, the DC were injected 24 h prior to the adoptive transfer of MBP-TCR T cells. This time delay is not required, as EAE also occurs when the DC and T cells are transferred on the same day (data not shown).
MBP-TCR CD4 T cells are detected in the CNS of animals with EAE

Because EAE is a disease of the CNS associated with the destruction of surrounding oligodendrocytes of the myelin sheath, disease symptoms should be accompanied by infiltration of encephalitogenic T cells into the brain and spinal cord. Using the animals shown in Fig. 4A, we examined brain and spinal cord for the presence of MBP-TCR CD4 T cells. Because the MBP-TCR contains a \( \alpha \) and \( \beta \) chain, we used a mAb specific for \( V_\beta 8.1/8.2 \) to detect the presence of transgenic T cells. We were able to visualize the presence of both CD4+ (Fig. 5A) and V\( \beta \)8.2+ (Fig. 5B) cells in the brains and spinal cords (data not shown) of mice with active EAE. To confirm that the T cell infiltration observed contained MBP-TCR CD4 T cells, we stained the tissue sections with a clonotypic mAb specific for the MBP-TCR (Fig. 5C). Mice from the same experiment which received medium-pulsed DC and did not exhibit signs of EAE showed little to no detectable infiltration of CD4+\, V\( \beta \)8.2+, or MBP-TCR+ (Fig. 5D, E, and F, respectively) cells in the brain. We have previously shown that in vitro-activated Ac 1–11-specific cloned T cells induced EAE upon adoptive transfer and...

**FIGURE 2.** DC pulsed with Ac 1–11 interact with MBP-TCR naive CD4 T cells in the lymph nodes, whereas medium-pulsed DC do not. BM-derived DC pulsed with medium alone or with Ac 1–11 were injected into the footpads of B10.PL mice along with the i.v. injection of naive MBP-TCR CD4 splenocytes. Prior to injection, the DC were labeled with the fluorescent dye DiO (green) and the T cells were labeled with DiI (red). After 24 h, the lymph nodes were harvested, and frozen sections were examined for interactions between DC and T cells. A and B show low power magnification, and C and D show high power magnification. Areas of direct interaction between Ac 1–11-pulsed DC are seen by the yellow fluorescence in B and by direct cell contacts in D. A and C are from mice that received DC pulsed with medium alone.

**FIGURE 3.** Splenic MBP-TCR tg T cells have a naive phenotype and can be primed in vivo with Ac 1–11-pulsed DC to become Th1 T cells. A, CD4+ T cells isolated from the spleen of a MBP-TCR tg mouse were cocultured with B10.PL splenocytes (H-2b) in the presence 1:10 dilutions of Ac 1–11 from 10 to 0.001 \( \mu \)g/ml. B, CD4+ T cells isolated from the spleen of a mouse 30 days following injection of Ac 1–11-pulsed DC and MBP-TCR CD4 T cells were cocultured with B10.PL splenocytes (H-2b) in the presence 1:4 dilutions of Ac 1–11 from 40 to 0.16 \( \mu \)g/ml. Proliferation (A and B) and subsequent secretion of IL-2 (A) or IFN-\( \gamma \) (B) within the same culture are shown. Proliferation was measured by \(^{38} \)H\( \)Tdr incorporation and presented as CPM on the left y-axis (A and B). Secretion of IL-2 was assayed using CTLL cells, as described in Materials and Methods, and shown on the right y-axis in A (\( \square \)) as U/ml. IFN-\( \gamma \) was assayed by ELISA, as described in Materials and Methods, and shown on the right y-axis in B (\( \square \)) as pg/ml. All individual data points were performed in duplicate and averaged.
were detected in the CNS of sick animals (12). Our ability to reproduce this result using our DC induction model is important for future studies on the dynamics of cell migration into the brain and on demyelination.

Discussion

EAE, an animal model of the human CNS autoimmune disease multiple sclerosis, can be induced actively or passively in genetically susceptible rodent strains. In mice, active induction of EAE requires immunization with either protein or peptide myelin Ags emulsified in CFA in combination with pertussis toxin injections. After 2 wk the first signs of disease, a limp tail and hind limb weakness, are observed. Depending on the mouse strain, the disease is either an acute monophasic disease course as observed in B10.PL mice (20) or relapsing and remitting as observed in SJL/J mice (21, 22). Passive induction of EAE requires the presolation of CD4+ T cells, generally of the Th1 phenotype, with Ag specificity for myelin Ags. Following in vitro activation, the T cells are adoptively transferred into irradiated recipient mice with disease symptoms occurring as early as 6–7 days later with transfer of large numbers of encephalitogenic T cells (12). The disease course is variable and can be acute or chronic monophasic or relapsing and remitting and is dependent on the number of T cells transferred and the mouse strain used in the study. Both models of EAE induction have been used extensively, with the active model most useful for studying the parameters involved in the initiation of EAE, and the passive model generally used in the study of the effector phase of EAE.

In this study, we describe a novel method of EAE induction using BM-derived DC pulsed with the Ac1–11 MBP peptide capable of interacting with and activating naive Ac1–11-specific T cells in vivo leading to EAE. We sought to develop a single model of EAE facilitating the study of the autoimmune process including initiation, effector, and recovery phases in one disease process. Building upon our previous work using B10.PL mice (20) and utilizing our Ac1–11-specific TCR tg mouse (13), we have successfully used DC as adjuvants to induce EAE. In our model, BM-derived DC were pulsed with Ac1–11 prior to s.c. injection into irradiated recipient mice, followed by i.v. injection of splenic naive CD4+ T cells. In concordance with our previous studies using B10.PL mice (20), we observed an acute monophasic disease course (Fig. 4B) followed by infiltration of MBP-TCR tg T cells into the brain parenchyma at a level we have previously observed only in passive EAE induction (Fig. 5) (12).

One of the major drawbacks to the study of EAE using active induction models is the necessity to use CFA as an adjuvant. CFA has been shown to preferentially induce Th1 immune responses over Th2 responses (23). This immune skewing can be beneficial, since a variety of studies have shown that the pathogenic T cell is typically of the Th1 phenotype (10), but has limitations in determining a role for Th2 cells. Th2 cells have been shown to be encephalitogenic under specific conditions (24) and are thought to be important in protection from EAE (10, 25, 26). Thus, the absence of CFA in our EAE model will allow a more precise study of the dynamics of cytokine profiles in EAE that have been suggested to switch from a Th1 pattern during the effector phase to a Th2 pattern during the recovery phase (10). In addition, we have eliminated the need for pertussis toxin, which inhibits G-protein function and has been shown to be immunomodulatory (27, 28).

In the mouse, the induction of EAE following the adoptive transfer of activated encephalitogenic T cells typically requires sublethal irradiation of the host animal. The irradiation is believed to facilitate the migration of T cells into the CNS, perhaps by up-regulating cytokines and adhesion molecules, leading to disease. Although irradiated animals were used in the experiments in Fig. 4, we were able to induce EAE in nonirradiated mice using our DC-based model of EAE induction (data not shown). Thus, DC alone in the absence of a stimulus provided by the mycobacteria in CFA, and in the absence of inflammatory signals provided by irradiation, can present self Ags resulting in the activation of self-reactive T cells leading to autoimmunity.

DC pulsed with tumor Ags have been used successfully in the treatment of a variety of animal tumor models (29). Although these studies have confirmed the efficient immune stimulatory activity of the adaptive immune response by DC, there remains much to learn about DC cell physiology. DC of BM origin are now known to reside in most tissues of the body. These DC are thought to be immature with the capacity for efficient and rapid Ag uptake leading to processing and presentation of Ags. In the case of foreign microbial Ags that activate DC, the DC are triggered to migrate to the draining lymph nodes via the afferent lymphatics. DC activation also initiates a rapid maturation process culminating with a mature DC with limited ability to take up and process Ag, high cell surface levels of MHC class II and B7-2, and a striking change in

FIGURE 4. Interactions between Ac1–11-pulsed DC and MBP-TCR CD4 naïve T cells in vivo leads to EAE. BM-derived DC were pulsed in vitro with medium alone or 100 μg/ml Ac1–11 and adoptively transferred into irradiated B10.PL mice. Twenty-four hours later, naïve splenic CD4 T cells isolated from MBP-TCR tg mice were adoptively transferred into the same animals. Animals were assessed daily for clinical signs of EAE. Animals were scored on a scale of 1–5 as described in Materials and Methods. Animals receiving DC pulsed with Ac1–11 are shown as filled circles, and animals receiving medium-pulsed DC are shown as open circles. Data shown in A and in B are from two separate experiments. Each group represents the average disease score of five mice, with the SE given.

Downloaded from http://www.jimmunol.org/ by guest on April 25, 2022
phenotype to a cell with numerous extended processes. This mature DC, now residing in the T cell zone, has a very slow turnover of peptide-bound MHC class II molecules on the cell surface (30). This DC maturation process was dramatically illustrated in vitro using BM-derived DC by tracking the location of MHC class II molecules from intracellular vesicles to the cell surface in combination with changes in morphology (17). Thus, the use of DC therapeutically requires the DC to be immature upon Ag pulsing. This is particularly important with protein Ags where peptide exchange on the cell surface is not a feasible mechanism (31). Although DC at various stages of maturation can present peptide Ags to T cell clones (32), only immature and not fully mature DC were shown to stimulate T cell clones when whole protein Ag was used (18). In this study, we used BM-derived DC that were enriched by replating overnight to allow cells of the monocyte lineage to strongly adhere. Although replating induces DC maturation, proliferation of the Ac1–11-specific MBP.TCR 31 clone occurred when the stimulating Ag was whole mMBP protein (Fig. 1C). This result demonstrates that the DC used in this study were capable of protein Ag uptake leading to processing and presentation.

Our use of T cells that are activated in vivo on peptide-loaded DC more closely mimics the actual series of events that occur between T cell priming and EAE induction in vivo, as compared with peptide immunization or the use of in vitro-activated T cell clones. In previous experiments, we have successfully induced EAE using a T cell clone expressing the Ac1–11-specific TCR from which the MBP tg mouse was generated (12). Although these experiments were successful, the use of T cell clones has limitations. The most problematic is the loss of encephalitogenic potential with continuous passage in culture as the result of phenotypic changes. Although phenotypic variations is not a concern in our model, a potential drawback to the use of CD4+ T cells from the MBP-TCR tg mouse is the presence of Ac1–11-specific Th1 memory cells that could rapidly expand and produce Th1 cytokines leading to EAE. In our model, this seems not to be the case since ~95% of the CD4+ T cells bear the Vβ8.2 TCR chain with only 1.2–1.5% of the starting tg T cell population of the memory phenotype (data not shown). This is consistent with a report by Linton, et al. (33), showing that CD4+ T cells in the AND TCR tg mouse are of a naive phenotype, even upon aging. In contrast, the memory cells detected showed little to no expression of the TCR tg. In addition, we were able to induce severe EAE with hind limb paralysis using Ac1–11-pulsed DC and transfer of only 4 × 10^6 Vβ8.2 MBP-TCR tg T cells, of which only 6 × 10^4 would be memory cells (data not shown). Although EAE has been shown to be induced following transfer of 1 × 10^5 activated encephalitogenic T cells, EAE onset was delayed until approximately day 28 and required both irradiation and injection of Bordetella pertussis (34). In addition, we have been able to induce EAE following the elimination of memory cells by cell sorting (data not shown).

A further expansion of our DC-based EAE induction model would be to induce EAE with Ac1–11-pulsed DC in the absence of transferred MBP-TCR T cells. To date, we have not been successful in inducing EAE in B10.PL mice without the transfer of MBP-TCR tg T cells (data not shown). We feel that a low precursor frequency of autoreactive T cells in the B10.PL mouse in combination with the reported low affinity of the Ac1–11 peptide for the...
I-A" MHC class II molecule (35) is not sufficient for the development of an immune response leading to autoimmunity. Another contributing factor is the transient presence of the peptide-pulsed DC in the lymph nodes, which decline in number 24 h following transfer (8). The use of CFA allows the slow constant release of Ag over a time frame of weeks. We feel that the transferred DC, although competent APC able to activate naive T cells, do not remain in the lymph nodes for a sufficient time to induce an autoimmune response. We are currently performing experiments using a modified Ac1-11 peptide with increased affinity for the I-A" MHC class II molecule combined with twice weekly transfers of DC to test our hypothesis.

A common strategy employed by a variety of investigators for preventing or diminishing the clinical symptoms of EAE is tolerance induction. Various mechanisms of tolerance induction have been utilized including the use of peptides (36, 37), thymic dendritic cells (38), and blocking of costimulatory molecules (39, 40). A likely common mechanism of these tolerogenic models is the prevention of the primary immune response. However, in multiple sclerosis patients, T cells with specificity for myelin Ags existing in the peripheral blood have an activated phenotype when compared to T cells from normal controls with comparable specificities (41–43). The long-term persistence of MBP-specific T cell clones in a multiple sclerosis patient suggests in vivo activation of the self reactive T cells (44). Thus, a mechanism of tolerance induction capable of rendering the myelin Ag-specific T cells unresponsive would be beneficial. We are currently using our DC EAE induction model to examine whether DC can deliver a tolerogenic signal to Ac1-11-specific naive T cells present prior to primary immunization and, more importantly, to primed CD4+ T cells after immunization. It is our hope that these and future studies using our DC EAE induction model will lead to therapies allowing the prevention or amelioration of multiple sclerosis.

Thus, our DC induction model of EAE allows the delivery of the encephalitogenic peptide Ac1-11 directly to the lymph node via migration of DC through the afferent lymphatics without the need for conventional adjuvants or in vitro T cell activation. Here, the DC encounter naive recirculating CD4+ T cells and arrest their migration, allowing Ag-specific activation of MBP-specific T cells into capable EAE effector cells.

Acknowledgments

We thank the W. M. Keck Foundation Biotechnology Resource laboratory for peptide synthesis, Craig Hammond for assistance with fluorescence microscopy, Jennifer Granata and Charles Annicelli III for assistance with the mice, Donna Sue Graeaser for assistance with the cloning of MBP.TCR 31, and Michae1 Carrithers for helpful discussions and assistance with brain anatomy.

References


