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Converting relapsing remitting to secondary progressive experimental allergic encephalomyelitis (EAE) by ultraviolet B irradiation

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Abstract

We induced experimental allergic encephalomyelitis (EAE) in SJL/J mice, an animal model for multiple sclerosis (MS), using myelin oligodendrocyte glycoprotein $(MOG)_{92–106}$ peptide, following ultraviolet (UV) irradiation. While all control mice developed relapsing–remitting (RR)-EAE, UV irradiation induced secondary progressive (SP)-EAE in some of the mice. Although mild demyelination was observed with T cell infiltration in RR-EAE, large demyelinating lesions developed in SP-EAE with massive macrophage and neutrophil infiltration and immunoglobulin deposition, but with little T cell infiltration. UV irradiation induced higher anti-MOG antibody responses. In SP-EAE, lymphoproliferative responses and interferon-γ production were decreased without alteration of interleukin-4.

Keywords: Autoantibodies; CNS demyelinating autoimmune diseases; Helper T-lymphocytes; Immunoglobulin isotypes; Photosensitivity disorders; Sunlight

1. Introduction

Although multiple sclerosis (MS) is believed to be an autoimmune demyelinating disease of the central nervous system (CNS), the precise etiology remains unclear. Epidemiologic studies show that the incidence of MS positively correlates with latitude (Kira, 2003; Libbey and Fujinami, 2002; McMichael and Hall, 1997). Although studies of migrant populations were performed to distinguish between genetic and environmental factors related to latitude (Visscher et al., 1977), there is no unanimous explanation for this latitude gradient. Interestingly, insulin-dependent diabetes mellitus, another organ specific autoimmune disease, also shows a similar latitude gradient, being higher with increasing distance from the equator (Diabetes Epidemiology Research International Group, 1988; Green et al., 1992; Ponsonby et al., 2002).

Solar ultraviolet (UV) radiation negatively correlates with latitude (De Fabo et al., 1990). UV light consists of a range of different wavelengths and is commonly subdivided into germicidal UV light (UVC, 100–280 nm), midrange UV light or sunburn UV light (UVB, 280–320 nm), and long wave UV light (UVA, 320–400 nm) (Orteu et al., 2001). UVB is immunosuppressive, particularly affecting cell mediated immunity, including delayed-type hypersensitivity (DTH) responses. McMichael and Hall (McMichael and Hall, 1997) hypothesized that the latitude gradient of MS prevalence may reflect differential UV induced suppression of autoimmune activity, since the autoimmune profile of MS is characterized by disturbances of T cell related functions that are affected by UVB.

However, there are several observations that do not fit this hypothesis. In MS, progression to a nonambulatory status or death was significantly greater among MS patients of the Los Angeles area of California than in the higher latitude-Seattle area of Washington (Detels et al., 1982). There is also a trend toward higher case mortality in patients from the Los Angeles area compared with those from the Seattle area (Malmgren et al., 1981). Nevertheless, there is a three-fold prevalence gradient in the opposite direction (higher prevalence in Washington State) (Visscher et al., 1977).

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The clinical, epidemiological, immunological, and pathological findings in progressive forms of MS are notably different from those described for relapsing–remitting (RR)-MS (Bashir and Whitaker, 1999; Suhy et al., 2000). For example, although three disease modifying therapies, interferon (IFN) β-1a, IFN β-1b, and glatiramer acetate, are approved for patients with RR-MS, treatment options for patients with progressive MS are few (Hartung et al., 2002). Based on the presence or absence of relapses and remission or progression of neurological deficits, the clinical course of MS can be divided into four forms: RR, primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR) (Lublin and Reingold, 1996). RR-MS is defined by disease relapses with full recovery or with sequelae. In contrast, PP-MS progresses continuously from the onset. RR disease is often followed by progression (SP-MS). The mechanism of the transition and differences between the forms are not well understood.

Using an encephalitogenic peptide from myelin oligodendrocyte glycoprotein (MOG), we previously established animal models that mimic the different forms of MS in two strains of *H-2*^{s} mice, SJL/J and A.SW (Tsunoda et al., 2000). We induced experimental allergic encephalomyelitis (EAE) in the presence or absence of supplemental *Bordetella pertussis* (*BP*). Although SJL/J mice developed RR-EAE whether *BP* was given or not, A.SW mice developed PP-EAE without *BP* supplementation and SP-EAE with *BP* supplementation. Histologically, SJL/J mice developed mild demyelinating disease with T cell infiltration, while A.SW mice developed large areas of plaque-like demyelination with immunoglobulin (Ig) deposition and polymorphonuclear (PMN) cell infiltration, but with minimal T cell infiltration. In A.SW mice without *BP*, high titer of serum anti-MOG antibody was detected and the anti-MOG IgG2a/IgG1 ratio correlated with survival times of mice. We hypothesized that, in A.SW mice, transition to a Th2 response favors the production of myelinotoxic antibodies, leading to progressive forms with early death. Our data using these models indicate that a single encephalitogen can induce either RR-, PP-, or SP-forms of demyelinating disease in hosts with immunologically different humoral immune responses.

In this paper, we tested the hypothesis that UV radiation, one of the most significant environmental factors, can alter the incidence and clinical course in an experimental animal model for MS. We induced EAE in SJL/J mice with MOG, following either a single dose or five doses of UV irradiation. Although mice receiving a single dose of UV irradiation showed a higher clinical disease incidence than mice without irradiation, those receiving five doses had a lower incidence of disease. Although no mice in the control group developed progressive EAE, UV irradiation induced SP-EAE in some of the mice. In SP-EAE, we found a large plaque-like demyelinating lesion consisting of massive numbers of macrophages and neutrophils with Ig deposition. Interestingly, T cell infiltration in the CNS, MOG-specific lymphoproliferation and IFN-γ production were decreased in SP-EAE versus RR-EAE. Our results could explain why increased solar irradiation decreases the incidence of MS in most cases, yet seems to lead to disease progression in other patients with MS.

2. Materials and methods

2.1. UV irradiation of mice

Female SJL/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Prior to irradiation the dorsal hair of the mice was removed with electric clippers and the skin further exfoliated using a commercial lotion hair remover. Irradiation was performed two days after hair removal. A bank of three Westinghouse FS-40 fluorescent sunlamps served as a source of UVB irradiation (Enioutina et al., 2002). Greater than 60% of the emitted energy was between 280 and 320 nm, whereas less than 1% was below 280 nm. Tube-to-target distance was 21 cm, and, under these conditions, the target received 2.9 J/m^2/s of UVB energy, as measured by an IL1350 radiometer, using a SED 240 UVB photodetector (International Light, Inc., Newburyport, MA). The total dose of UVB received by the animals in a 34-min period was approximately 6.0 kJ/m^2. Groups of mice were irradiated once (UV×1) or daily over five consecutive days (UV×5). All control mice were shaved and sham irradiated.

2.2. EAE induction and scoring

One hour after the last dose of UV irradiation, mice were sensitized in the base of the tail with 100 nmol of $MOG_{92–106}$ peptide (DEGGYTCFFRDHSYQ) (Core Facility, University of Utah Huntsman Cancer Institute, Salt Lake City, UT) (Amor et al., 1994) in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, MI) by the subcutaneous route. Mice were weighed six times per week and observed for clinical signs for 4–6 months. Classical EAE signs were assessed according to the following criteria (Tsunoda et al., 1998): 0=no clinical disease; 1=loss of tail tonicity; 2=mild hind leg paresis; 3=moderate hind leg paralysis; 4=complete paraplegia; and 5=quadriplegia, moribund state or death. Mice immunized with $MOG_{92–106}$ have been shown to have not only classical signs of EAE but also a second clinical phenotype, ataxic form (Greer et al., 1996; Tsunoda et al., 2000), which commenced with mice turning their heads or bodies to one side (Score 1 or 2 depending on the degree to which the head was turned) with or without a waddling gait. Score 3=mice continuously rolled by twisting their bodies or rotated laterally

in a circle; 4=mice could not stand but would lay on their side with or without rolling; and 5=moribund state or death.

RR-EAE was defined as follows: clearly defined disease attacks with full recovery or with sequelae and residual deficit upon recovery; periods between disease relapses characterized by a lack of disease progression (Lublin and Reingold, 1996). Since the disease did not progress, no mice with RR-EAE died (mortality was 0% by the definition). In this experiment, mice with a single disease episode (monophasic EAE) were also categorized as RR-EAE group. SP-EAE was defined as follows: initial RR disease course followed by progression with or without occasional relapses, minor remissions, and plateaus. When the mice had switched from RR-EAE to SP-EAE, the disease progressively worsened. Therefore, all mice with SP-EAE became moribund and were euthanized or died (mortality was 100%). We categorized mice as SP-EAE when mice became moribund or died.

2.3. Histology

Mice were euthanized with halothane when moribund, or after the four to six month observation period. We perfused mice with phosphate buffered saline (PBS), followed with a 4% paraformaldehyde solution. Brains were divided into five coronal slabs and spinal cords into 10–12 transverse slabs, and tissues were embedded in paraffin. Four micrometer thick tissue sections were stained with Luxol fast blue for myelin visualization. Histologic scoring was performed as described previously (Tsunoda et al., 1998, 2000). Brain sections were scored for meningitis (0=no meningitis; 1=mild cell infiltrates; 2=moderate cell infiltrates; 3=severe cell infiltrates), perivascular cuffing (0=no perivascular cuffing; 1=1–10 lesions; 2=11–20 lesions; 3=2130 lesions; 4=3140 lesions; 5=over 40 lesions), and demyelination (0=no demyelination; 1=mild demyelination; 2=moderate demyelination; 3=severe demyelination). For scoring spinal cord sections, each spinal cord section was divided into quadrants: the anterior funiculus, the posterior funiculus, and each lateral funiculus. Any quadrant containing meningitis or demyelination was given a score of 1 in that pathologic class. The total number of positive quadrants for each pathologic class was determined, then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for each pathologic class. An overall pathologic score was also determined by giving a positive score if any lesions were present in the quadrant.

2.4. Immunohistochemistry and lectin histochemistry

T cells were immunostained with anti-CD3ε antibody (following trypsinization, 1:30 dilution, Dako, Carpinteria, CA) (Mason et al., 1989; Tsunoda et al., 2000, 2003) and Ig deposition was detected with biotin conjugated anti-mouse IgG (H+L) antibody (1:200 dilution, Jackson ImmunoResearch Laboratories, West Grove, PA) (Tsunoda et al., 2000). Microglia and macrophages were identified by biotinylated *Ricinus communis agglutinin* (RCA) I (1:5000 dilution, Vector Laboratories, Burlingame, CA) (Suzuki et al., 1988; Tsunoda et al., 2003). Each were visualized using the avidin–biotin peroxidase complex (ABC) technique with 3,3′-diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) as the chromogen. Sections were analyzed independently by *Image-Pro*® Plus version 4.5.1 (Media Cybernetics, Silver Spring, MD) (Tsunoda et al., 2000).

2.5. Serum anti-MOG antibody assay

Blood was obtained from MOG immunized mice when sacrificed. We used an enzyme-linked immunosorbent assay (ELISA) to measure the level of anti-MOG antibody as previously described (Tsunoda et al., 1999, 2000). Ninety-six well plates were coated overnight with $MOG_{92–106}$ peptide. After blocking, serial dilutions of sera were added to the plates and incubated for 90 min. After washing, a peroxidase conjugated anti-mouse IgG (H+L) (Life Technologies, Gaithersburg, MD), IgG1, IgG2b antibody (Caltag Laboratories, Burlingame, CA) or IgG2c (Southern Biotechnology Associates, Birmingham, AL) (Martin et al., 1998) antibody was added for 90 min. Bound antibodies were visualized using *o*-phenylenediamine dihydrochloride (Sigma-Aldrich) as the chromogen. The plates were read at 492 nm on a Titertek Multiskan Plus MK II spectrophotometer (Flow Laboratories, McLean, VA).

2.6. Lymphoproliferation assay

Inguinal lymph nodes and spleens were removed and pooled, and mononuclear cells (MNCs) were isolated with Histopaque®-1083 (Sigma-Aldrich). A volume of 100 μl containing 2×10^5 cells in RPMI 1640 (Mediatech, Herndon, VA) supplemented with 1% glutamine (Mediatech), 1% antibiotics (Mediatech), 50 μM 2-mercaptoethanol (Sigma-Aldrich) and 10% fetal bovine serum (Invitrogen) was added to each well of 96-well plates. This was followed with 100 μl of $MOG_{92–106}$ peptide such that the final concentration of peptide was 50 μg/ml. The cells were then cultured for four days at which time each well was pulsed with 1 μCi of tritiated thymidine (PerkinElmer Life Sciences, Boston, MA), and cells were cultured for another 24 h. Cultures were harvested onto filters using a multiwell cell harvester (Molecular Devices, Sunnyvale, CA) and ^3H incorporation was determined (Tsunoda et al., 1998). All cultures were performed in triplicate and results were expressed as stimulation indexes (SI, experimental cpm/control cpm).

2.7. Cytokine assay

Spleen and lymph node cells were cultured at 2×10^6 cells/ml in 6 well plates in the presence or absence of concanavalin A (Con A) (5 μg/ml, Sigma-Aldrich), or $MOG_{92–106}$ peptide (50 μg/ml). Culture supernatants were harvested 48 h after stimulation. We measured IFN-γ and interleukin (IL)-4 using the ELISA system, OptEIA™ Set

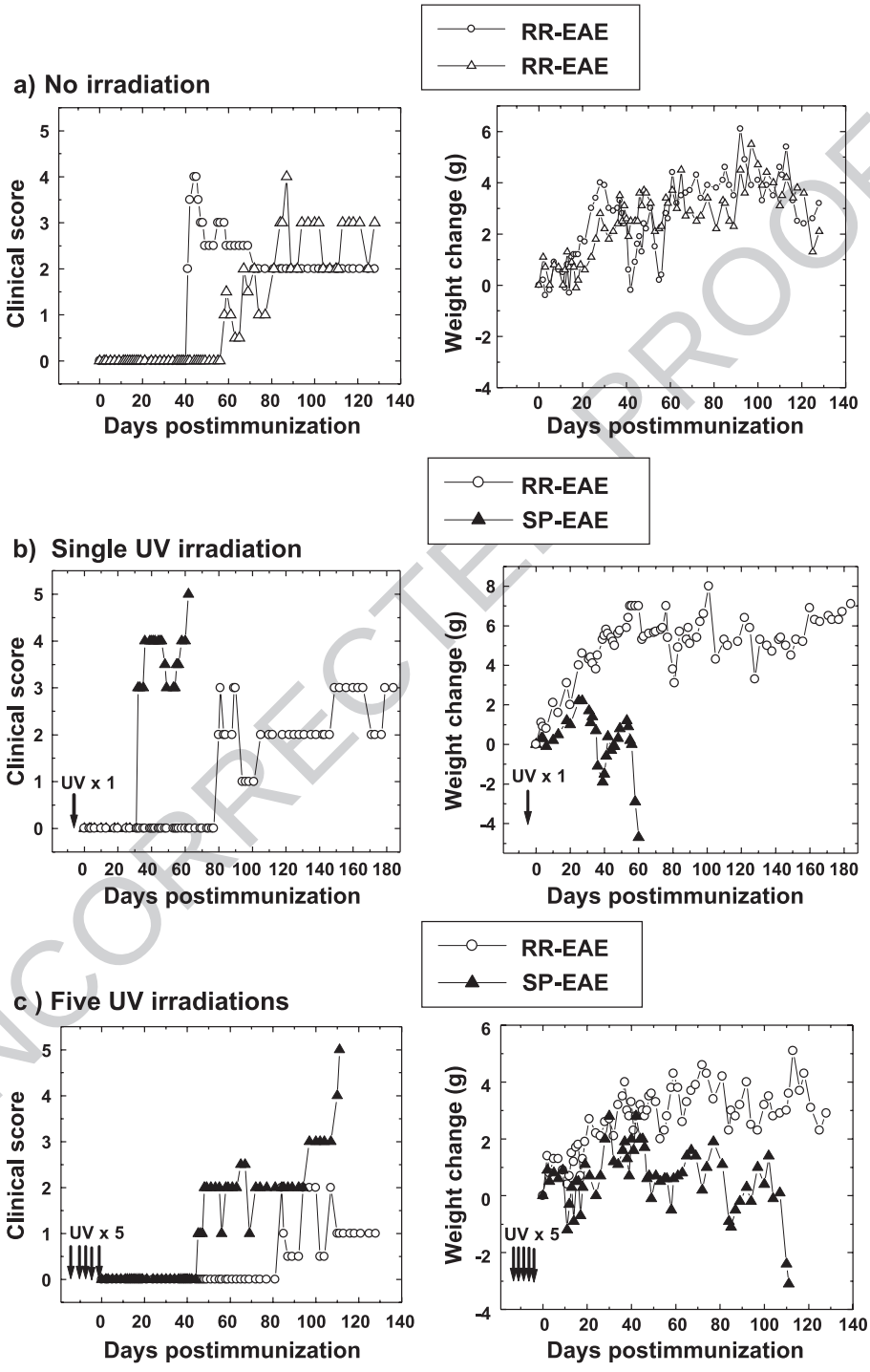

Fig. 1. Clinical course of MOG-induced EAE following UV irradiation. Mice were immunized with $MOG_{92–106}$ peptide in CFA with no irradiation (a), a single dose of UV irradiation (b) or five doses of UV irradiation (c). Mice were observed for clinical signs (left) and weight changes (right). (a) In the control group without UV irradiation, 10 of 17 mice developed EAE, all of which had a RR disease course (open circle and triangle). (b) In 22 mice receiving a single dose of UV irradiation, 13 mice had a RR disease course (open circle) and 6 mice initially showed RR disease followed by progression, SP-EAE (closed triangle). (c) In 21 mice receiving five doses of UV irradiation, 6 mice had RR-EAE (open circle) and 3 mice developed SP-EAE (closed triangle). Shown is the clinical course of two representative animals from each group in three independent experiments. Statistics of the experiments were shown in Table 1.

(BD Biosciences, San Diego, CA), according to the manufacturer's instructions (Tsunoda et al., 1999).

3. Results

3.1. UV irradiation induces progressive disease

In three separate experiments, we irradiated mice with UVB once or five times before MOG immunization (Fig. 1). Sham irradiated control mice were immunized with MOG. In all groups, mice showed either classic paralytic signs, ataxic type of clinical signs or no clinical signs. In the control group, 59% of mice developed clinical signs of RR-EAE, and no mice in this group developed progressive EAE (Table 1, Fig. 1a). Although mice receiving a single dose of UV irradiation showed an increase in disease incidence (86%) versus mice without irradiation, mice receiving five doses of UV irradiation had a lower incidence of clinical disease (43%). While most mice that developed clinical signs had a RR disease course, about one-third of mice receiving a single dose of UV irradiation and a smaller portion of mice receiving five doses had a progressive disease course (SP-EAE, Fig. 1b,c). Mice receiving a single dose of UV irradiation had a significantly more severe clinical disease than those mice receiving five doses of irradiation or no irradiation ($p<0.05$, ANOVA, Table 1).

3.2. A single dose of UV irradiation results in severe neuropathology

After a four to six month observation period, we compared the presence or absence of lesions in brains and spinal cords of mice immunized with MOG among groups with or without UV irradiation (Table 1). All mice receiving a single dose of UV irradiation had lesions in the brain and spinal cord, compared with 13 of 15 mice not receiving UV irradiation. These included several animals that exhibited no evidence of clinical disease. Mice receiving five doses of UV irradiation showed a significantly lower incidence of histological disease; only 8 of 20 (40%) mice had lesions in the spinal cord (Table 1) ($p<0.05$ and $p<0.01$, compared with none and a single dose of UV irradiation, respectively).

We also compared the severity of the neuropathologic lesions among the groups, using mean pathology scores in the brain and spinal cord. Mice receiving a single dose of UV irradiation (Fig. 2a,b, UV×1) had higher meningitis and demyelination scores than those in mice receiving five doses of UV irradiation (UV×5) or no irradiation. There was no statistical difference in the mean neuropathology scores between the groups of mice receiving none versus five doses of UV irradiation. The lower mean pathology score of the latter group was most likely due to the fact that 57% of the group of mice showed no clinical signs despite 14% of the mice developed SP-EAE. Thus, we divided each group with different doses of UV irradiation into three subgroups based on clinical signs: (1) mice with no clinical signs, (2) RR-EAE or (3) SP-EAE. In each subgroup, there was no statistical difference in the pathology scores between mice receiving none, a single or five doses of UV irradiation. Then, we combined all data from control and UV irradiated groups and compared the pathology scores between mice with no clinical signs, RR-EAE or SP-EAE. Mice with RR-EAE or SP-EAE had higher brain pathology scores than those mice with no clinical signs (Fig. 2c, $p<0.01$, ANOVA, no difference between RR-EAE versus SP-EAE). In the spinal cord, mice with SP-EAE had the highest pathology scores in all categories (Fig. 2d, $p<0.01$, compared with RR-EAE and asymptomatic groups, ANOVA), while those of mice with RR-EAE were statistically different from those of mice with no clinical signs ($p<0.01$, ANOVA).

t1.1 Table 1
t1.2 Clinical diseases and neuropathology of mice immunized with MOG following UV irradiation

Treatment[a]	Clinical disease[b]	SP-EAE/mice[c] (Mortality)	Clinical score[d]	Neuropathology/mice[e]		
				Brain	Spinal cord	CNS
No treatment	10/17 (59%)	0/17 (0%) *	1.5 ± 0.4	13/15 (87%)	12/15 (80%)	13/15 (87%)
UV×1	19/22 (86%) **	6/22 (27%) *	2.7 ± 0.4[f]	18/19 (89%)	17/19 (89%) ** *	19/19 (100%) *
UV×5	9/21 (43%) **	3/21 (14%)	1.3 ± 0.4	15/20 (75%)	8/20 (40%) **	15/20 (75%) *

t1.3 [a] Mice were immunized with myelin oligodendrocyte glycoprotein $(MOG)_{92–106}$ peptide in complete Freund's adjuvant (CFA) following no treatment, a single dose of (UV×1) or five doses of (UV×5) ultraviolet irradiation.
t1.4 [b] Number (percentage) of mice with clinical signs/total number of mice examined.
t1.5 [c] Number (percentage) of mice with secondary progressive experimental allergic encephalomyelitis (SP-EAE)/total number of mice examined. All mice with SP-EAE became moribund and were euthanized or died, while none of mice with RR-EAE or no clinical signs died during the observation period.
t1.6 [d] Values are mean maximum clinical scores±SEM.
t1.7 [e] Number (percentage) of mice with histological lesions in the brain, the spinal cord or the central nervous system (CNS, brain and spinal cord lesions combined)/total number of mice examined.
t1.8 [f] $p<0.05$, ANOVA, compared with those of no treatment and UV×5.
t1.9 * $p<0.05$; ** $p<0.01$ by a chi-square test.

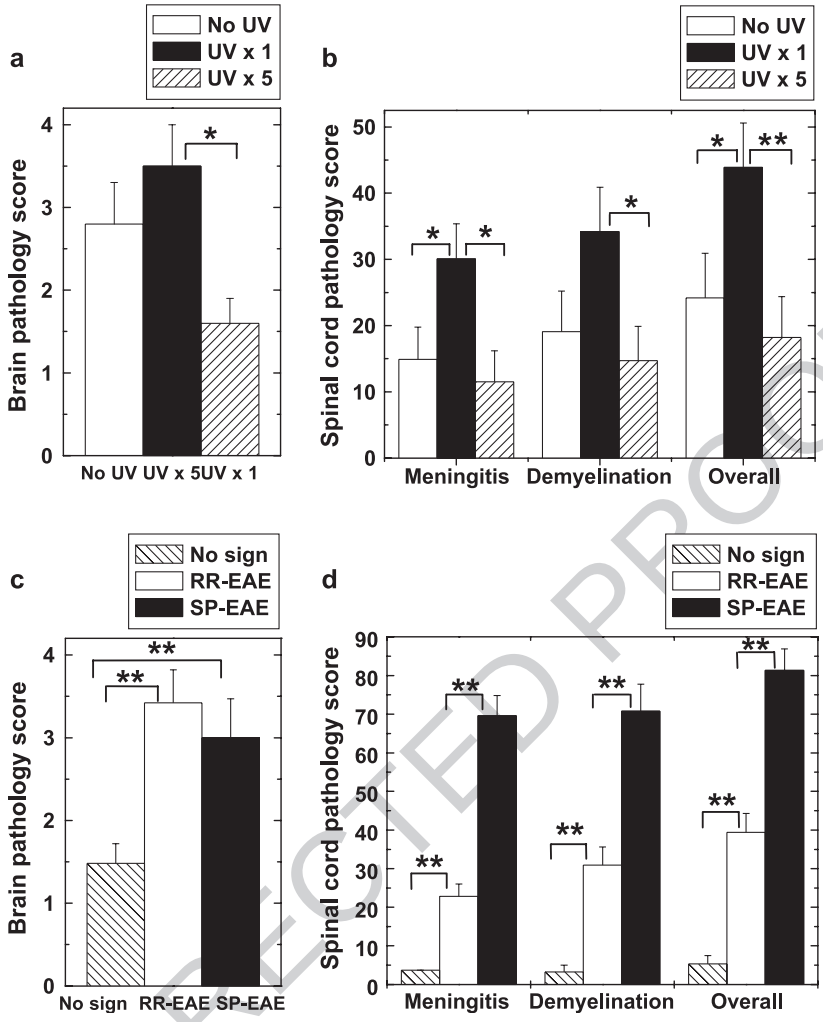

Fig. 2. Neuropathology score of MOG induced EAE mice following UV irradiation. Mice were sensitized with $MOG_{92–106}$ following a single dose of UV irradiation (UV×1) or five doses of UV irradiation (UV×5) or with no UV irradiation (No UV). (a) Mice receiving a single dose of UV irradiation (closed) had a higher mean brain pathology score than the control group without UV irradiation, while mice with five doses of UV irradiation showed a lower brain score than the control group. (b) A single dose of UV irradiation resulted in higher meningitis, demyelination, and overall pathology scores than the control group or the group receiving five doses of UV irradiation (*p<0.05; **p<0.01, ANOVA). Each treatment group consisted of 15–20 mice. We also compared pathology scores between mice with different clinical signs. (c) Mice with RR-EAE (open) or SP-EAE (closed) had statistically higher brain pathology scores than mice without clinical signs (hatched) (**p<0.01, ANOVA). (d) In the spinal cord, mice with SP-EAE had the highest neuropathology scores, statistically (**p<0.01, ANOVA). Values are the means±SEM of pooled results from three independent experiments.

3.3. Demyelination and composition of the inflammatory lesions differ between RR-EAE versus SP-EAE

The extent of demyelination and the cell types composing inflammatory infiltrates differed between mice with RR disease versus mice with progressive disease, even within the same group of mice receiving the same dose of UV irradiation. In mice with RR-EAE, we found mild areas of demyelination with perivascular and meningeal MNC infiltration within the internal capsule and in the white matter of the cerebellum, the brain stem and the spinal cord (Fig. 3a,b). This pattern of lesion distribution was found in mice with RR-EAE, whether UV irradiation was provided or not. In contrast, mice with SP-EAE developed large plaque-like regions of demyelination with macrophage and PMN infiltration (Fig. 3c,d). Large demyelinating areas were not accompanied by perivascular cuffing, and MNC infiltrates were mild in both meningeal and perivascular spaces. There was no difference in the composition of the cellular infiltrates in mice with SP-EAE induced by a single dose of UV irradiation compared with those receiving five doses. The anatomic distribution of lesions in SP-EAE was similar to that seen in RR-EAE.

3.4. T cells, macrophages and Ig deposition in the CNS

To further characterize the lesions, we compared T cell, macrophage infiltration and Ig deposition in the CNS of mice with SP-EAE and RR-EAE. We immunostained and enumerated T cells in meninges, perivascular spaces and parenchyma of the CNS, using CD3 as a T cell specific

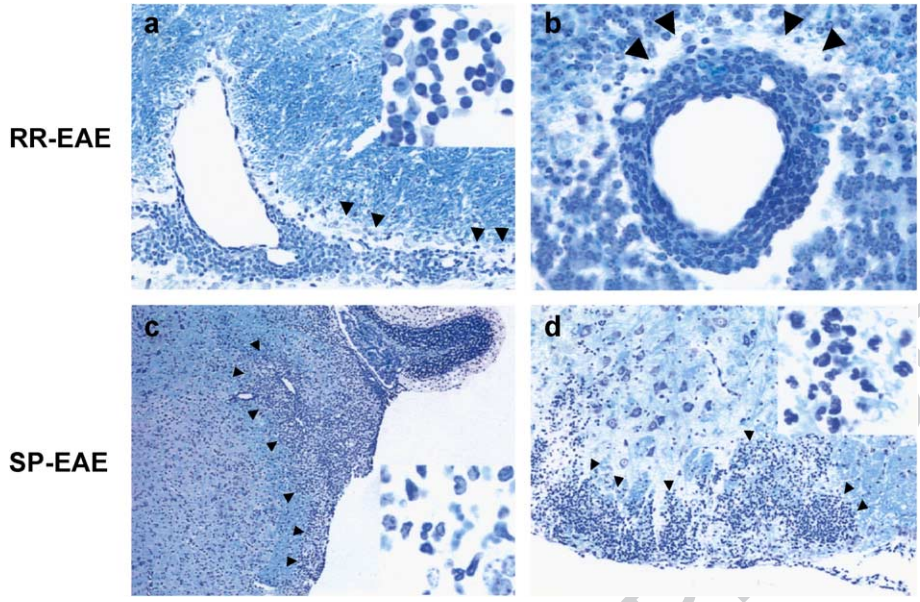

Fig. 3. Neuropathology of MOG immunized mice with RR-EAE (a, b) or with SP-EAE (c, d). Mice with RR-EAE developed meningitis (a) and perivascular cuffing (b) with mild subpial and perivascular demyelination (arrowhead), respectively. The infiltrates were predominantly composed of MNCs (a, inset). In UV-irradiated mice with SP-EAE, we found large demyelinating lesions with predominantly macrophage and PMN infiltration (c) and predominant PMN infiltration in the white matter (d). (a) midbrain, (b) cerebellum, (c) cerebellopontine angle, (d) pontine base. (a, c, d) A single dose of UV irradiation. (b) No UV irradiation. Luxol fast blue stain (a–d). Magnification: a, ×110; b, ×230; c, ×20; d, ×60; inset, ×360.

marker. In RR-EAE, T cells predominated in meningeal and perivascular lesions (Figs. 4 and 5d). Such T cells were also seen infiltrating the CNS parenchymal demyelinating lesions. In contrast, in mice with SP-EAE, T cells comprised only a small proportion of the cellular infiltrates in both meningeal and perivascular spaces, and very few T cells were detected in parenchymal demyelinating lesions (Fig. 5c). The actual numbers of T cells in the meninges, perivascular space, and parenchyma were significantly lower in mice with SP-EAE than in mice with RR-EAE ($p<0.01$, Fig. 4). We combined all the data from control and UV irradiated groups and compared numbers of T cells in cellular infiltrates between mice with RR-EAE versus mice with SP-EAE. [Note: There was no difference in T cell infiltrates among mice with RR-EAE, whether UV irradiation was given or not. We found no difference in T cell numbers in infiltrates in mice with SP-EAE given a single dose versus five doses of UV irradiation (data not shown)].

Macrophages and activated microglia were detected by RCA I lectin histochemistry (Suzuki et al., 1988; Tsunoda et al., 2003). In mice with RR-EAE, small numbers of RCA I^+ cells were sporadically detected in the white matter (Fig. 5f). On the other hand, in SP-EAE mice, demyelinating lesions were heavily infiltrated by rounded RCA I^+ phagocytes (Fig. 5e).

Antibody mediated pathology has been shown to play an effector role in tissue destruction and disease progression of MOG induced EAE (Linington et al., 1989; Tsunoda et al., 2000). Using immunohistochemistry for mouse Ig, we detected mild deposition of Ig in meninges and perivascular lesions of mice with RR-EAE (Fig. 5h). We found some meningeal infiltrates were intensely Ig positive, suggesting the presence of plasma cells. In the parenchyma, however, no significant Ig deposition was seen in mice with RR-EAE. In contrast, in half of mice with SP-EAE, we detected intense Ig deposition around parenchymal cell infiltrates and at the plaque margins (Fig. 5g).

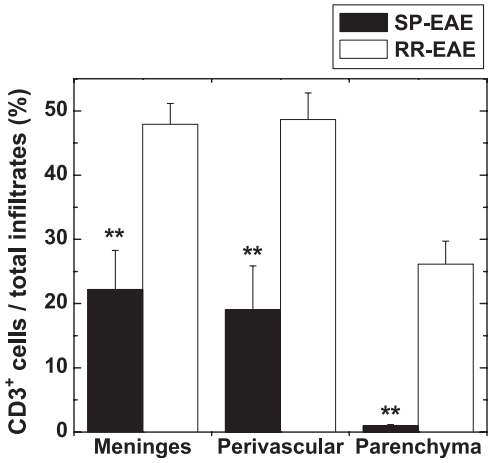

Fig. 4. Percentage of T cells in the total cell infiltrates in mice with SP-EAE and RR-EAE. We detected T cells in brain and spinal cord sections with anti-CD3 antibody. In mice with RR-EAE, $CD3^+$ T cells predominated in meningeal and perivascular infiltrates, and a significant number of T cells were also seen infiltrating into the CNS parenchyma. In SP-EAE, a significantly lower number of T cells were detected in meninges, perivascular cuffing and parenchyma, compared with those in RR-EAE (**$p<0.01$, t-test). Data represent the mean percentage of T cells in cell infiltrates (the number of $CD3^+$ cells/total number of cells in infiltrate) ±SEM of CNS sections from 78 lesions in SP-EAE and 134 lesions in RR-EAE.

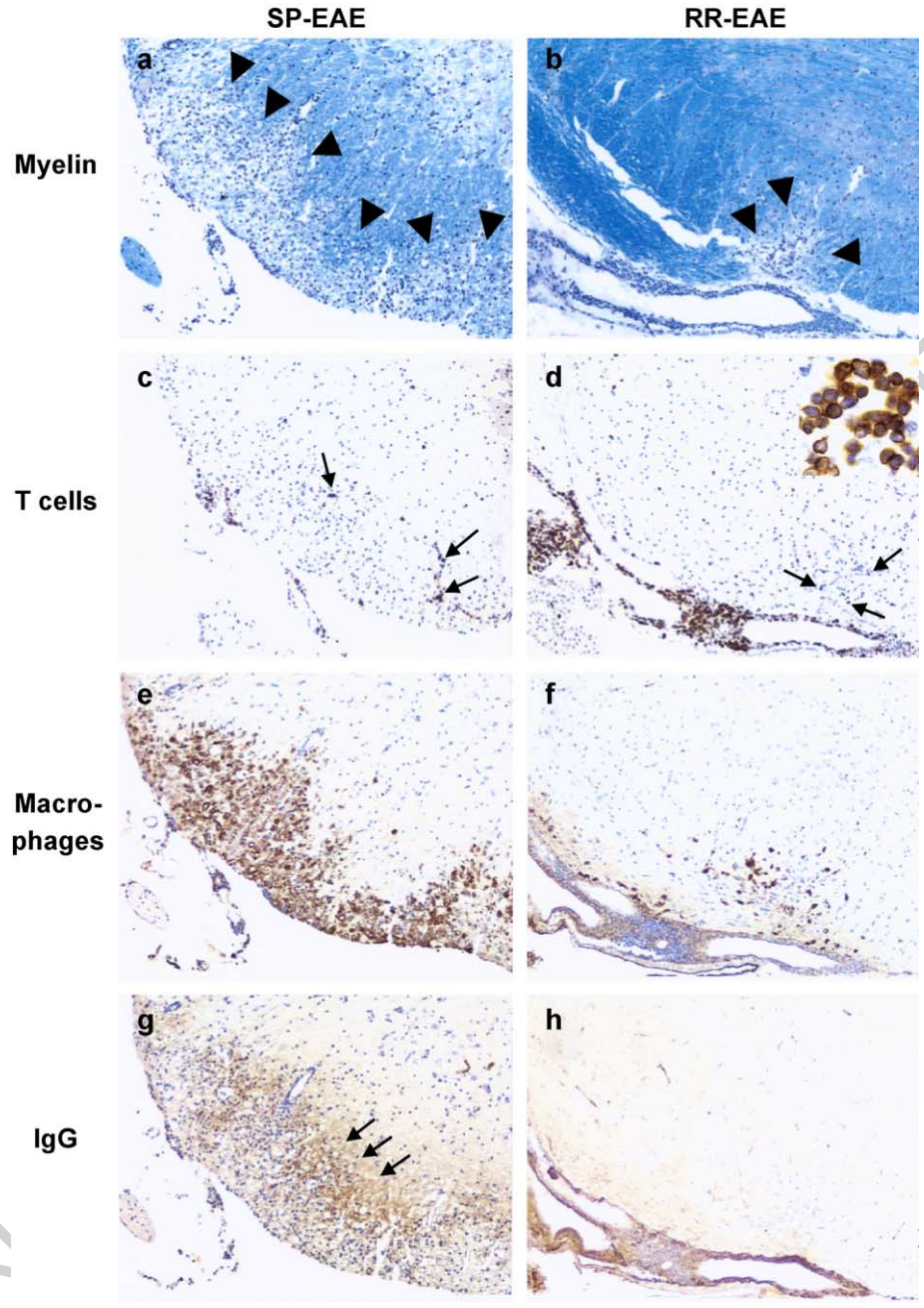

Fig. 5. Demyelination (a, b), T cell infiltration (c, d), macrophage/microglia infiltration (e, f) and immunoglobulin (Ig) deposition (g, h) in MOG-induced EAE mice with a single dose of UV irradiation. In SP-EAE, large subpial demyelinating lesions with mild meningeal cell infiltration were seen. In the demyelinating lesions, although only a few T cells were detected (c), massive infiltration of the plaque tissue by RCA I^+ macrophages and microglia (e), and Ig deposition were seen at the plaque margin (g). In RR-EAE, despite significant MNC infiltration in meninges, we detected only mild demyelination (b). T cells predominated in meningeal infiltrates (d), and macrophages were sporadically seen in demyelinating lesions (f), while Ig was detected only in meninges (h). Luxol fast blue stain (a, b). Immunohistochemistry against CD3 (c, d) and mouse Ig (g, h). RCA I lectin histochemistry (e, f). Magnification: a–h, ×50; inset, ×300.

3.5. UV radiation increases MOG specific antibody

Since we detected Ig deposition in mice with progressive disease, we tested whether serum anti-MOG antibody titers correlated with the clinical and pathological findings. As seen in Fig. 6, we detected higher serum anti-MOG IgG, IgG1, and IgG2c (formerly IgG2a of *Igh-1b* allotype) (Jouvin-Marche et al., 1989; Martin and Lew, 1998; Parsons et al., 1986), and IgG2b responses in UV irradiated groups than in the no irradiation group. Although mice with SP-EAE tended to have high levels of anti-MOG antibody in their sera [anti-MOG IgG (H+L); 0.737±0.365 (mean $OD_{492\ nm}$ at 1:1024 dilution±SEM)], a few mice showed lower anti-MOG antibody responses than those of asymp-

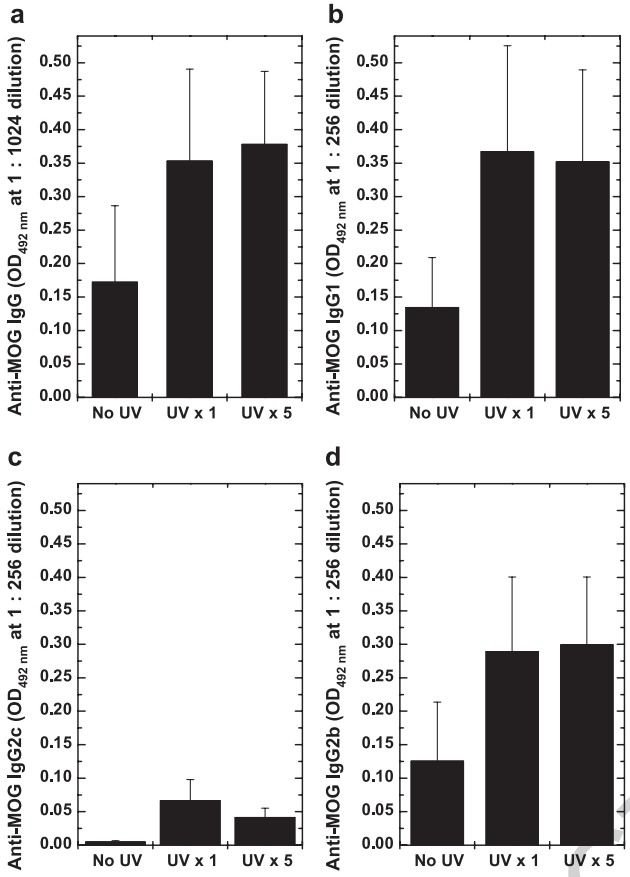

Fig. 6. Serum anti-MOG antibody responses in MOG-induced EAE mice with no irradiation (No UV) or following a single dose of UV irradiation (UV×1) or five doses of UV irradiation (UV×5). Mice receiving a single dose or five doses of UV irradiation had increased anti-MOG whole IgG (a), IgG1 (b), IgG2c (c) and IgG2b (d) responses. Values are mean optical density (OD) ±SEM of pooled results from three independent experiments. Each treatment group consisted of 14–18 mice.

tomatic mice. We also did not see a correlation between spinal cord pathology scores and anti-MOG IgG titers in mice with SP-EAE (r=0.341, p=0.616). Since there was a large range of antibody levels among individual mice even within the same group, there was no statistical difference between groups.

3.6. MOG specific lymphoproliferative responses are decreased in SP-EAE

We examined lymphoproliferative responses to $MOG_{92–106}$ using MNCs isolated from lymph nodes and spleens. In mice with RR-EAE, slightly higher proliferation was seen by MNCs from symptomatic mice than those from mice without clinical signs (mean SI±SEM: symptomatic, 3.04±0.56; asymptomatic, 1.14±0.1, p<0.01). Among the mice with RR-EAE, no significant differences were observed between groups with or without UV treatment (data not shown). In contrast, we did not detect MOG specific lymphoproliferation in mice with SP-EAE (mean SI±SEM: 1.07±0.17). This suggests that MOG specific T cell responses correlate with clinical signs in RR-EAE, but not in SP-EAE.

3.7. IFN-γ, but not IL-4, production decreases in SP-EAE

Although active humoral immune responses were detected in mice with SP-EAE, cellular immune responses appeared to be suppressed in this group. Since alterations in cytokine production could result in an imbalance of humoral and cellular immune responses, we compared production of IFN-γ versus that of IL-4 by spleen or lymph node cells from mice with either SP-EAE or RR-EAE. Regardless of whether mice were treated with UV or not, high levels of IFN-γ were detected from Con A stimulated cultures from all groups of mice with RR-EAE (Fig. 7). In contrast, spleen cells from mice with SP-EAE produced only low levels of IFN-γ. In contrast, we found no difference in IL-4 between

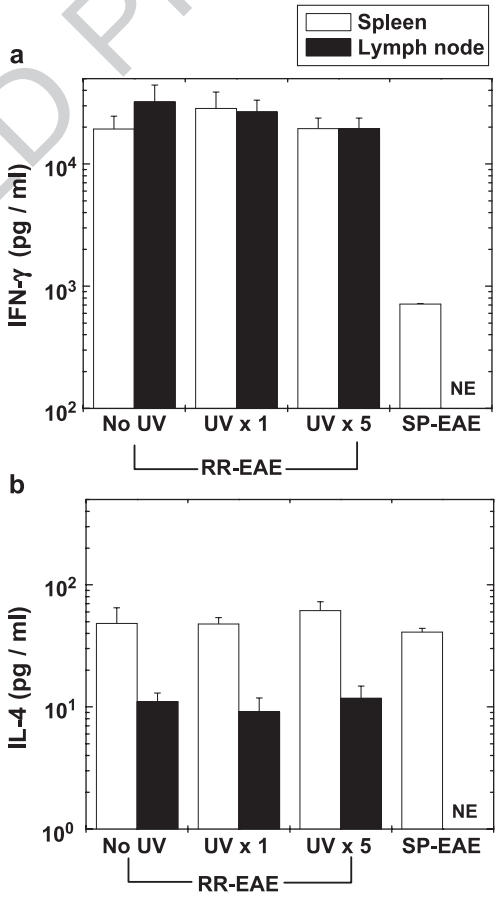

Fig. 7. IFN-γ (a) and IL-4 (b) production in MOG immunized mice with RR-EAE with no irradiation (No UV) or following a single dose of UV irradiation (UV×1) or five doses of UV irradiation (UV×5) versus mice with SP-EAE induced by UV irradiation. The cytokine concentrations of the supernatants from Con A stimulated MNCs of spleens or regional lymph nodes were measured by an ELISA. (a) IFN-γ production was low in SP-EAE, while high levels of IFN-γ production were detected in all groups of mice with RR-EAE. (b) In contrast, no difference in IL-4 production was seen between all RR-EAE mice versus SP-EAE mice. Since lymph nodes of SP-EAE mice were atrophic, it was not possible to examine cytokine production (NE).

mice with RR-EAE versus those with SP-EAE. In all groups, significant cytokine production was seen only in the supernatant of Con A stimulated cultures, and not in MOG stimulated or unstimulated cultures. Due to severe lymph node atrophy, we were not able to examine cytokine responses using lymph node cells from mice with SP-EAE. Nevertheless, the results suggest that the imbalance of Th1 versus Th2 immune responses might alter humoral and cellular immune responses and contribute to disease progression.

4. Discussion

Here we demonstrate that multiple doses of UV irradiation can decrease the incidence of EAE, yet, induced SP-EAE in some of the mice. This observation is consistent with an epidemiological finding where the extent of UV irradiation negatively correlates with the prevalence of MS, yet might contribute to disease progression (Detels et al., 1982). Similar to our results, Hauser et al. (1984) has shown that UV treatment of SJL/J mice before immunization prevented the development of acute EAE (between days 13 and 20 post immunization) induced using mouse spinal cord homogenate. Unlike our experiments, they did not see the development of SP-EAE. This is most likely due to the difference of the type of EAE. Hauser et al. (1984) induced acute EAE with spinal cord homogenate and followed mice for a shorter period of time; whereas, our model uses $MOG_{92–106}$ and is chronic EAE without acute disease.

The neuropathology of mice with SP-EAE induced using UV irradiation was similar to that observed in A.SW mice with SP-EAE (Tsunoda et al., 2000). In both cases, we found large plaque-like areas of demyelination with large numbers of infiltrating PMNs and macrophages and Ig deposition with very few T cells present in the CNS. This is in contrast to the perivascular and subpial demyelination observed in mice with RR-EAE, which was associated with a predominant T cell component. Immunologically, as with SP-EAE in A.SW mice, SP-EAE in UV treated SJL/J mice had higher anti-MOG immune responses but lower MOG-specific lymphoproliferation and lower Con A induced IFN-γ production than SJL/J mice with RR-EAE. These results suggest anti-myelin antibodies, rather than myelin-specific T cells, contribute to the disease progression.

It has been shown that UV irradiation has immunosuppressive effects on cell mediated immune responses, such as contact hypersensitivity and DTH responses (Kripke, 1994). In contrast, UV irradiation does not suppress humoral immune responses and shifts antigen specific IgG isotype responses towards a Th2 response, evidenced by an increase in the production of the IgG1 antibodies and a decrease in IgG2a antibodies (Enioutina et al., 2002). In our experiments, UV irradiation of mice led to an overall increase in anti-MOG antibody, particularly those of IgG1.

A number of studies on the role of the relative Th1/Th2 balance in organ-specific autoimmune disease have proposed a pathogenic role of Th1 cells and either protective or innocuous role of Th2 cells (Shevach, 1999). Under certain conditions, however, Th2 cells may produce more severe pathology, leading to fatal disease. Lafaille et al. (1997) demonstrated that myelin basic protein (MBP) specific Th2 cells can induce EAE when adoptively transferred into immunocompromised RAG knockout mice or into αβ T cell deficient mice, while MBP specific Th1 cells, but not Th2 cells, causes EAE upon transfer into normal mice. Interestingly, this Th2 cell mediated EAE is similar to our MOG induced progressive EAE in which both models have a late onset of disease and PMN cell infiltration. In addition, Genain et al. (1996) demonstrated hyperacute progressive EAE in MOG immunized marmosets, in which Th2 responses seemed to be preferentially magnified, with exacerbated demyelination due to the production of pathogenic autoantibodies.

Similar results were observed in a mouse model for insulin-dependent diabetes mellitus, another apparent organ specific autoimmune disease (Pakala et al., 1997). When Th1 or Th2 cells stimulated with islet cells were adoptively transferred into immune competent NOD mice, only mice receiving Th1 cells became diabetic. However, adoptive transfer of Th1 cells or Th2 cells was able to induce diabetes in two types of immune compromised recipients: NOD mice lacking αβ T cells (NOD $C\alpha^{-/-}$) and NOD.*scid* mice. The Th1 lesion was comprised of peri-insulitis and insulitis with β cell apoptosis and little exocrine inflammation. In Th2 lesions, PMNs and macrophages infiltrated not only the islets but also the exocrine tissue with significant necrosis and abscess formation, while T cells made up only a small portion of these infiltrates.

In the above studies, immunocompromised (or immunosuppressive) phenotypes of the host seemed to contribute to the development of a Th2 mediated disease. In our UV induced SP-EAE studies, we also have evidence of systemic immunosuppression. As shown in Fig. 7, we found a decrease in IFN-γ production, with production of sustained levels of IL-4, by spleen MNCs from mice with SP-EAE. In these mice, we found severe atrophy of lymphoid organs, including thymus, lymph node and spleen. The mean weight of spleens from mice with SP-EAE was significantly decreased compared to spleens from mice with RR-EAE [$p<0.01$ by t-test, mean spleen weight (g/mouse)±SEM: SP-EAE, 0.048±0.003; RR-EAE, 0.144±0.018]. This is similar to the finding in A.SW mice with PP-EAE and SP-EAE, where we previously observed a severe atrophy of lymphoid organs with T cell depletion (Tsunoda et al., 2000) (Tsunoda I and Fujinami RS, in preparation). Both in a current study using UV-irradiated SJL/J mice and the previous study using MOG immunized A.SW mice, only mice with SP-EAE developed the severe atrophy of lymphoid organs with a decrease in IFN-γ (Tsunoda et al., 2000). In contrast, SJL/J mice with RR-EAE, whether

receiving UV or not, produced the large amounts of IFN-γ with no atrophy of lymphoid organs. Similarly, in A.SW mice with $MOG_{92–106}$, large amounts of IFN-γ with no lymphoid atrophy were seen during the latent period and at the disease onset. The severe decrease in IFN-γ was seen only during the disease progression in A.SW mice. Thus, among MOG immunized mice, only the mice whose cytokine imbalance reached at a certain critical level seemed to develop a progressive disease. Therefore, as in other models, the immunosuppression or immunomodulation seen during the chronic stage, particularly suppression of Th1 type responses, could contribute to disease progression.

Although we do not know the precise mechanism by which UV irradiation modulated immune responses in our model, UV irradiation has been demonstrated to alter immune responses through multiple mechanisms (Kripke, 1994). Overall, exposure to UV radiation correlates with a predominance in cytokines that promote Th2 immune responses at the expense of Th1 immune responses and that may result in immunomodulation. UV irradiation can induce production of cytokines, including IL-4, IL-10, tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β, (Hart et al., 2000; Schwarz et al., 2000), in a variety of cell types, including keratinocytes, macrophages, dendritic cells, Th2 cells, T regulatory (Tr) cells, mast cells, neutrophils, natural killer T (NKT) cells, and fibroblasts (Hart et al., 1998, 2000; Moodycliffe et al., 2000; Schwarz et al., 2000; Teunissen et al., 2002; Ullrich, 1996). UV irradiation can also modify antigen presenting cell function in the irradiated skin (Kripke, 1994) and induce antigen specific suppressor cells (Ts) cells (Daynes et al., 1986). In addition, UV radiation catalyzes production of vitamin D_3, which can regulate immune responses and modulate the clinical signs of MS and EAE (Hayes et al., 1997).

Another possible mechanism for how UV irradiation alters the clinical course of EAE is induction of apoptosis in the irradiated skin and/or lymphoid organs. UV irradiation can induce apoptotic cells with Fas and FasL expression in the skin (Furukawa, 2003; Hill et al., 1999) and uptake of apoptotic cells by lipopolysaccharide (LPS) stimulated macrophages results in the enhanced secretion of IL-10 and decreased secretion of TNF-α, IL-1β and IL-12 (Voll et al., 1997). In addition, intravenous injections of syngeneic apoptotic cells into mice induce polyclonal activation of autoantibody production (Mevorach et al., 1998). In our current experiments, we have confirmed that UV irradiation induces apoptosis in irradiated skin as determined by TUNEL staining and upregulation of Fas and FasL expression in the epidermis (data not shown). We also found many apoptotic cells in atrophic lymphoid organs in SP-EAE. Consistent with involvement of apoptotic cells in the development of SP-EAE, in preliminary experiments, we determined that intravenous injection of syngeneic apoptotic cells into MOG sensitized SJL/J mice led to a switch from RR-EAE to SP-EAE, similar to what is reported here (Tsunoda I and Fujinami RS, in preparation).

In our model, the balance of cytokines leading to cellular and/or humoral immune responses seemed to be important. During the induction phase, Th1 type immune responses can contribute to disease onset, as shown in other Th1 mediated organ specific autoimmune diseases, including classical monophasic and RR-EAE. During the chronic phase, however, Th2 or humoral immune responses might play an important role in disease progression. In the latter, SP-EAE has characteristics of systemic autoimmune diseases, rather than organ specific autoimmune diseases: (1) production of autoantibody, rather than T cells, with imbalance of cytokines seems to play a pathogenic role in disease progression (Tsunoda et al., 2000); and (2) lesions were found in multiorgans, including the lung, the liver, and the kidney (Tsunoda and Fujinami, unpublished data). Interestingly, UV radiation has been reported to aggravate symptoms of patients with systemic lupus erythematosus (SLE), a prototypic systemic autoimmune disease characterized by autoantibody production (Cohen, 1999). In SLE and other systemic autoimmune diseases, Th2 cytokines seem to play important roles in promoting autoimmunity (Froncek and Horwitz, 2002). Although the available evidence is largely anecdotal, systemic disease, including fatigue, joint pain and fever, can be increased several months after SLE patients received an increased amount of UV radiation during the summer months, in conjunction with an increase in cutaneous lesions, including rash to unexposed skin (Krause et al., 1997; Orteu et al., 2001; Wysenbeek et al., 1989).

Based on the present data, we propose the following scenario for UV induced SP-EAE. UV irradiation modulates the balance between Th1 versus Th2 responses and increases anti-myelin antibody responses, contributing to exacerbation of disease. At the same time, however, in some mice, five doses of UV irradiation could suppress Th1 immune responses. Since Th1 immune responses are required for disease onset, but not for progression, this could result in a lower incidence of EAE in this group. Among the irradiated mice with RR-EAE, apoptosis was induced in lymphoid organs by unknown mechanisms. This might suppress Th1 type immune responses and skew immune responses further toward Th2 type responses. This would favor the production of myelinotoxic antibody, leading to disease progression.

Our current experiments provide a clinical prediction of the potential benefits (decreased incidence of disease) and detrimental effects (induction of progressive disease) that are associated with immunomodulation, in this case, by the exposure of skin to solar or UV radiation. Caution should be used before the adoption of such an immune deviation therapy in organ specific autoimmunity, including MS. In addition, epidemiological research will be required to see how UV irradiation affects disease activity of patients with different clinical courses of MS, namely, RR-, SP-, PP- and PR-MS.

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References

Amor, S., Groome, N., Linington, C., Morris, M.M., Dornmair, K., Gardinier, M.V., Matthieu, J.M., Baker, D., 1994. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. J. Immunol. 153, 4349–4356.

Bashir, K., Whitaker, J.N., 1999. Clinical and laboratory features of primary progressive and secondary progressive MS. Neurology 53, 765–771.

Cohen, P.L., 1999. Systemic autoimmunity. In: Paul, W.E. (Ed.), Fundamental Immunology, Fourth Edition. Lippincott-Raven Publishers, Philadelphia, PA, pp. 1067–1088.

Daynes, R.A., Samlowski, W.E., Burnham, D.K., Gahring, L.C., Roberts, L.K., 1986. Immunobiological consequences of acute and chronic UV exposure. Curr. Probl. Dermatol. 15, 176–194.

De Fabo, E.C., Noonan, F.P., Frederick, J.E., 1990. Biologically effective doses of sunlight for immune suppression at various latitudes and their relationship to changes in stratospheric ozone. Photochem. Photobiol. 52, 811–817.

Detels, R., Clark, V.A., Valdiviezo, N.L., Visscher, B.R., Malmgren, R.M., Dudley, J.P., 1982. Factors associated with a rapid course of multiple sclerosis. Arch. Neurol. 39, 337–341.

Diabetes Epidemiology Research International Group, 1988. Geographic patterns of childhood insulin-dependent diabetes mellitus. Diabetes 37, 1113–1119.

Enioutina, E.Y., Visic, D.M., Daynes, R.A., 2002. The induction of systemic and mucosal immunity to protein vaccines delivered through skin sites exposed to UVB. Vaccine 20, 2116–2130.

Froncek, M.J., Horwitz, D.A., 2002. Cytokines in the pathogenesis of systemic lupus erythematosus. In: Wallace, D.J., Hahn, B.H. (Eds.), *Dubois' Lupus Erythematosus*, Sixth Edition. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 187–203.

Furukawa, F., 2003. Photosensitivity in cutaneous lupus erythematosus: lessons from mice and men. J. Dermatol. Sci. 33, 81–89.

Genain, C.P., Abel, K., Belmar, N., Villinger, F., Rosenberg, D.P., Linington, C., Raine, C.S., Hauser, S.L., 1996. Late complications of immune deviation therapy in a nonhuman primate. Science 274, 2054–2057.

Green, A., Gale, E.A., Patterson, C.C., 1992. Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE study. Lancet 339, 905–909.

Greer, J.M., Sobel, R.A., Sette, A., Southwood, S., Lees, M.B., Kuchroo, V.K., 1996. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. J. Immunol. 156, 371–379.

Hart, P.H., Grimbaldeston, M.A., Swift, G.J., Jaksic, A., Noonan, F.P., Finlay-Jones, J.J., 1998. Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. J. Exp. Med. 187, 2045–2053.

Hart, P.H., Grimbaldeston, M.A., Jaksic, A., Tan, J.E., Swift, G.J., Hosszu, E.K., Halliday, G.M., Finlay-Jones, J.J., 2000. Ultraviolet B-induced suppression of immune responses in interleukin-4−/− mice: relationship to dermal mast cells. J. Invest. Dermatol. 114, 508–513.

Hartung, H.-P., Gonsette, R., König, N., Kwiecinski, H., Guseo, A., Morrissey, S.P., Krapf, H., Zwingers, T., 2002. Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. Lancet 360, 2018–2025.

Hauser, S.L., Weiner, H.L., Che, M., Shapiro, M.E., Gilles, F., Letvin, N.L., 1984. Prevention of experimental allergic encephalomyelitis (EAE) in the SJL/J mouse by whole body ultraviolet irradiation. J. Immunol. 132, 1276–1281.

Hayes, C.E., Cantorna, M.T., DeLuca, H.F., 1997. Vitamin D and multiple sclerosis. Proc. Soc. Exp. Biol. Med. 216, 21–27.

Hill, L.L., Ouhtit, A., Loughlin, S.M., Kripke, M.L., Ananthaswamy, H.N., Owen-Schaub, L.B., 1999. Fas ligand: a sensor for DNA damage critical in skin cancer etiology. Science 285, 898–900.

Jouvin-Marche, E., Morgado, M.G., LeGuern, C., Voegtle, D., Bonhomme, F., Cazenave, P.-A., 1989. The mouse Igh-1^{a} and Igh-1^{b} H chain constant regions are derived from two distinct isotypic genes. Immunogenetics 29, 92–97.

Kira, J., 2003. Multiple sclerosis in the Japanese population. Lancet Neurol. 2, 117–127.

Krause, I., Shraga, I., Molad, Y., Guedj, D., Weinberger, A., 1997. Seasons of the year and activity of SLE and Behcet's disease. Scand. J. Rheumatol. 26, 435–439.

Kripke, M.L., 1994. Ultraviolet radiation and immunology: something new under the sun-presidential address. Cancer Res. 54, 6102–6105.

Lafaille, J.J., Van de Keere, F., Hsu, A.L., Baron, J.L., Haas, W., Raine, C.S., Tonegawa, S., 1997. Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J. Exp. Med. 186, 307–312.

Libbey, J.E., Fujinami, R.S., 2002. Are virus infections triggers for autoimmune disease? Clin. Microbiol. Newsl. 24, 73–76.

Linington, C., Morgan, B.P., Scolding, N.J., Wilkins, P., Piddlesden, S., Compston, D.A., 1989. The role of complement in the pathogenesis of experimental allergic encephalomyelitis. Brain 112, 895–911.

Lublin, F.D., Reingold, S.C., 1996. Defining the clinical course of multiple sclerosis: results of an international survey. [National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis]. Neurology 46, 907–911.

Malmgren, R.M., Dudley, J.P., Visscher, B.R., Valdiviezo, N.L., Clark, V.A., Detels, R., 1981. Mortality in persons with multiple sclerosis in the Seattle and Los Angeles areas. JAMA 246, 2042–2045.

Martin, R.M., Lew, A.M., 1998. Is IgG2a a good Th1 marker in mice? Immunol. Today 19, 49.

Martin, R.M., Brady, J.L., Lew, A.M., 1998. The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice. J. Immunol. Methods 212, 187–192.

Mason, D.Y., Cordell, J., Brown, M., Pallesen, G., Ralfkiaer, E., Rothbard, J., Crumpton, M., Gatter, K.C., 1989. Detection of T cells in paraffin wax embedded tissue using antibodies against a peptide sequence from the CD3 antigen. J. Clin. Pathol. 42, 1194–1200.

McMichael, A.J., Hall, A.J., 1997. Does immunosuppressive ultraviolet radiation explain the latitude gradient for multiple sclerosis? Epidemiology 8, 642–645.

Mevorach, D., Zhou, J.L., Song, X., Elkon, K.B., 1998. Systemic exposure to irradiated apoptotic cells induces autoantibody production. J. Exp. Med. 188, 387–392.

Moodycliffe, A.M., Nghiem, D., Clydesdale, G., Ullrich, S.E., 2000. Immune suppression and skin cancer development: regulation by NKT cells. Nat. Immunol. 1, 521–525.

Orteu, C.H., Sontheimer, R.D., Dutz, J.P., 2001. The pathophysiology of photosensitivity in lupus erythematosus. Photodermatol. Photoimmunol. Photomed. 17, 95–113.

Pakala, S.V., Kurrer, M.O., Katz, J.D., 1997. T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. J. Exp. Med. 186, 299–306.

Parsons, M., Herzenberg, L.A., Stall, A.M., Herzenberg, L.A., 1986. Mouse imunoglobulin allotypes. In: Weir, D.M., Herzenberg, L.A., Blackwell, C., Herzenberg, L.A. (Eds.), Handbook of Experimental Immunology, Fourth edition. Blackwell Scientific Publications, Oxford, England, pp. 97.1–97.17.

Ponsonby, A.-L., McMichael, A., van der Mei, I., 2002. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. Toxicology 181–182, 71–78.

Schwarz, A., Beissert, S., Grosse-Heitmeyer, K., Gunzer, M., Bluestone, J.A., Grabbe, S., Schwarz, T., 2000. Evidence for functional relevance of CTLA-4 in ultraviolet-radiation-induced tolerance. J. Immunol. 165, 1824–1831.

Shevach, E.M., 1999. Organ-specific autoimmunity. In: Paul, W.E. (Ed.), Fundamental Immunology, Fourth Edition. Lippincott-Raven Publishers, Philadelphia, PA, pp. 1089–1125.

Suhy, J., Rooney, W.D., Goodkin, D.E., Capizzano, A.A., Soher, B.J., Maudsley, A.A., Waubant, E., Andersson, P.B., Weiner, M.W., 2000. ^{1}H MRSI comparison of white matter and lesions in primary progressive and relapsing–remitting MS. Mult. Scler. 6, 148–155.

Suzuki, H., Franz, H., Yamamoto, T., Iwasaki, Y., Konno, H., 1988. Identification of the normal microglial population in human and rodent nervous tissue using lectin-histochemistry. Neuropathol. Appl. Neurobiol. 14, 221–227.

Teunissen, M.B.M., Piskin, G., di Nuzzo, S., Sylva-Steenland, R.M.R., de Rie, M.A., Bos, J.D., 2002. Ultraviolet B radiation induces a transient appearance of IL-4^{+} neutrophils, which support the development of Th2 responses. J. Immunol. 168, 3732–3739.

Tsunoda, I., Kuang, L.-Q., Tolley, N.D., Whitton, J.L., Fujinami, R.S., 1998. Enhancement of experimental allergic encephalomyelitis (EAE) by DNA immunization with myelin proteolipid protein (PLP) plasmid DNA. J. Neuropathol. Exp. Neurol. 57, 758–767.

Tsunoda, I., Tolley, N.D., Theil, D.J., Whitton, J.L., Kobayashi, H., Fujinami, R.S., 1999. Exacerbation of viral and autoimmune animal models for multiple sclerosis by bacterial DNA. Brain Pathol. 9, 481–493.

Tsunoda, I., Kuang, L.-Q., Theil, D.J., Fujinami, R.S., 2000. Antibody association with a novel model for primary progressive multiple sclerosis: induction of relapsing–remitting and progressive forms of EAE in H2^{S} mouse strains. Brain Pathol. 10, 402–418.

Tsunoda, I., Kuang, L.-Q., Libbey, J.E., Fujinami, R.S., 2003. Axonal injury heralds virus-induced demyelination. Am. J. Pathol. 162, 1259–1269.

Ullrich, S.E., 1996. Does exposure to UV radiation induce a shift to a Th-2-like immune reaction? Photochem. Photobiology 64, 254–258.

Visscher, B.R., Detels, R., Coulson, A.H., Malmgren, R.M., Dudley, J.P., 1977. Latitude, migration, and the prevalence of multiple sclerosis. Am. J. Epidemiol. 106, 470–475.

Voll, R.E., Herrmann, M., Roth, E.A., Stach, C., Kalden, J.R., Girkontaite, I., 1997. Immunosuppressive effects of apoptotic cells. Nature 390, 350–351.

Wysenbeek, A.J., Block, D.A., Fries, J.F., 1989. Prevalence and expression of photosensitivity in systemic lupus erythematosus. Ann. Rheum. Dis. 48, 461–463.