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Facilitation of experimental allergic encephalomyelitis by irradiation and virus infection: role of inflammatory cells

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Abstract

Infection with an avirulent strain of Semliki Forest virus (SFV-A7) facilitates the development of experimental allergic encephalomyelitis (EAE) in a genetically resistant BALB/c mouse strain. Irradiation which is necessary for EAE induction caused a decrease in the total number of lymphocytes and an increase in $CD4^+/CD8^+$ T cell ratio in the spleen of BALB/c mice. EAE induction increased the ratio further until clinical and histological signs of EAE appeared. Entry of perivascular $CD4^+$ and $CD8^+$ cells preceded the onset of clinical signs and the appearance of MAC-1^+ cells in the central nervous system (CNS). In the acute phase of EAE, cellular infiltrates, which were sparse, consisted mainly of MAC-1^+ cells and a few $CD4^+$ and $CD8^+$ cells. Inflammatory cells gradually disappeared during the recovery phase. SFV-A7 infection after irradiation and EAE induction did not significantly change the $CD4^+/CD8^+$ ratio in the spleen or in the CNS infiltrates but enhanced the entry of inflammatory cells into the CNS. Similar perivascular cell influx was also seen in untreated mice infected with SFV-A7. We conclude that observed rapid reduction of splenic mononuclear cells and increase of the $CD4^+/CD8^+$ T cell ratio caused by irradiation prior EAE induction are early crucial events in disease induction in this resistant strain of mice. SFV-A7 infection, which further facilitates the development of EAE, does not induce immunoregulatory changes but provides its effect by enhancing the entry of inflammatory cells into the CNS. The combination of these two mechanisms thus effectively breaks the natural resistance against EAE in this genetically resistant mouse strain.

Keywords: Experimental allergic encephalomyelitis; Semliki Forest virus infection; Irradiation; T cell subset

1. Introduction

Acute experimental allergic encephalomyelitis (EAE) is a model for a T cell-mediated autoimmune disease that shares features with the human disease multiple sclerosis (MS) (Raine, 1984). EAE can be induced in susceptible rats and mice by active immunization or by passive transfer of $CD4^+$ encephalitogenic T cells to naive recipients (Sedgwick et al., 1987). In an acute EAE lesion of genetically susceptible SJL mouse, T and B lymphocytes are found in perivascular and meningeal areas and within CNS parenchyma. Infiltrating $CD4^+$ and $CD8^+$ cells are sparse (Traugott et al., 1985) although T lymphocytes are essential for the initiation of EAE. Myelin basic protein (MBP)-activated $CD4^+$ T cells from SJL/J mice with EAE can transfer the disease to naive recipients (Pettinelli and McFarlin, 1981). It has been shown that activated MBP-specific $CD4^+$ T cells cause typical EAE in naive Lewis rats but the same treatment results in haemorrhagic cellular infiltrates in CNS of rats immunosuppressed chemically or by irradiation (Sedgwick et al., 1987). This suggests that the cells may mediate the effect by inducing vascular damage (Sedgwick et al., 1987). In susceptible DA rats an increase in $CD4^+/CD8^+$ T cell ratio in blood after induction of EAE occurs before the appearance of clinical disease. This increase is due to the generation of a high number

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of antigen-specific $CD4^+$ T cells after sensitization with neuroantigen (Vukmanovic et al., 1990).

The role of $CD8^+$ T cells in murine EAE is unclear. There is evidence that $CD8^+$ cells in the murine EAE are responsible for disease suppression (Lider et al., 1989) and for resistance to a second induction of EAE after recovery from the first episode (Jiang et al., 1992). In Lewis rat, however, long-term depletion of $CD8^+$ T cells did not change the induction, recovery or subsequent resistance phases of the disease (Sedgwick, 1988). Furthermore, intraperitoneal injection of mice with a monoclonal antibody (mAb) specific for the mouse CD8 antigen neither alters the disease course nor leads to an increase in severity of histological or neurological signs of EAE in SJL mice (Sriram and Carroll, 1988). In the mutant $CD8^-/^-$ mice the EAE onset and susceptibility were unchanged. Acute EAE was milder with fewer deaths but lead more often to a chronic disease with a higher frequency of relapses. This suggests that $CD8^+$ T lymphocytes have a role both as effectors and as regulators in EAE (Koh et al., 1992).

B lymphocytes are also present in the inflammatory lesion late during acute EAE in SJL mice but their presence is restricted to perivascular cuffs (Sriram et al., 1982). Similarly, a small number of macrophages are found in a later phase of the developing EAE lesion. This suggests that other mechanisms than receptor-mediated phagocytosis are operative in myelin destruction in SJL/J mouse EAE which is in contrast to acute MS lesion with numerous macrophages (Traugott et al., 1985).

Virus infections can also result in demyelination. Theiler's virus produces demyelinative lesions with extensive mononuclear inflammatory cell infiltrates where demyelination is not primarily cytolytic but dependent on the host immune response (Dal Canto and Rabinowitz, 1982). Rats infected with JHM virus develop T-cell sensitization to both virus and myelin basic protein, resulting in demyelination (Watanabe et al., 1983), whereas certain mutants of the JHM virus produce demyelination in mice by direct infection of the oligodendrocytes (Stohlman and Weiner, 1981). Thus, virus-induced demyelination can either be cytolytic or immunomediated.

We have earlier described a BALB/c model of EAE in which genetically resistant BALB/c mice are rendered susceptible to the induction of EAE by whole body irradiation (Wu et al., 1988). Injection with mouse spinal cord homogenate results in development of EAE between 21 and 28 days after injection in 15–30% of mice. Infection with a non-lethal Semliki forest virus mutant (SFV-A7) after irradiation and EAE induction further increases the frequency of clinical disease to 70% of mice. The clinical signs appear 5–9 days earlier than in mice with EAE induction only.

Virus infections can change the absolute and relative numbers of different subsets of T cells (Rubin et al., 1981). If the balance between effector and regulatory T cells is disturbed, autoimmunity may be enhanced. This could be one of the mechanisms of EAE enhancement by virus infection. To study this possibility we have determined the changes in spleen cell subset profiles in our EAE model. The cellular composition of developing EAE lesions in CNS was also studied and different EAE induction protocols were compared.

2. Materials and methods

2.1. Mice

BALB/c (Harlan Sprague Dawley, Inc., USA) and SJL/OLA/HSD (Harlan Olac Limited, UK) female, 6–8-week-old mice were obtained from the Central Animal Laboratory, University of Turku, Finland.

2.2. Virus

An avirulent mutant of Semliki Forest virus (SFV-A7) was obtained from Dr. H.E. Webb (Neurology Unit, Department of Neurology, Rayne Institute, St. Thomas' Hospital, London, UK) and grown in a BALB/c mouse brain cell line (MBA-1) previously established in our laboratory. Virus was titrated in another BALB/c brain cell line (MBA-13) using a standard plaque assay. The virus was stored in 1-ml aliquots at $-70°C$. Virus was used at 1×10^6 plaque-forming units (PFU)/mouse injected intraperitoneally in 100 μl of sterile phosphate-buffered saline (PBS).

2.3. Monoclonal antibodies

For the flow cytometry (FACS) analyses of lymphocyte subsets in spleens, phycoerythrin (PE)-conjugated rat anti-mouse L3T4 (CD4 helper/inducer) mAb and fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse Lyt.2 (CD8 suppressor/cytotoxic) mAb were purchased from Becton Dickinson, Mountain View, CA.

Rat hybridoma cell clone GK1.5 (produces antibody reactive with T cell surface antigen L3T4 = CD4 helper/inducer T cells), clone 2.43 (produces antibody reactive with Lyt-2.2 = CD8 surface antigen, suppressor/cytotoxic T cells), clone M1/70.15.11.5 (produces antibody reactive with macrophage-granulocyte-specific surface antigen MAC-1 = CD11b), were obtained from the American Type Culture Collection (ATCC). Rat anti-mouse CD4, CD8 and MAC-1 monoclonal antibodies were purified from culture supernatants and used for immunohistochemical analysis of lymphocyte

subsets in frozen sections. FITC-conjugated affinity-purified goat antibody to rat IgG was used as a secondary antibody in FACS analysis of MAC-1-positive cells.

2.4. Induction of EAE in BALB/c and SJL mice

BALB/c mice were irradiated with 3.5 Gy using linear accelerator (4 MeV photons) at the Department of Radiation Therapy and Oncology of the Turku University Hospital, Turku, Finland.

Lyophilized mouse spinal cord homogenate (MSCH) in suspension containing 3 mg of MSCH in 50 μl of sterile PBS and 50 μl of complete Freund's adjuvant (CFA) was injected subcutaneously in hind footpads of BALB/c mice 2 days after 3.5 Gy whole body irradiation. Pertussis toxin (100 ng in 100 μl sterile PBS, Islet-activating protein, List Biological Laboratories, Inc., USA) was injected intravenously via the tail vein at days 1 and 3 after administration of the neuroantigen. A group of mice received an additional 10^6 PFU SFV-A7 intraperitoneal infection 7 days after induction. A group of control mice received 2 days after irradiation and two pertussis toxin injections at days 1 and 3 after the administration of CFA without MSCH in CFA at day 0. EAE in SJL mice was induced as in BALB/c mice but without irradiation.

2.5. Clinical evaluation

Mice were followed daily after EAE induction and scored from 0 to 4 according to the following scale: 0, normal; 1, fur ruffing; 2, tail atonia, slight hind limb paralysis; 3, hind limb paralysis; 4, moribund state.

2.6. Flow cytometry analysis

Time course studies of spleen cell subsets were done as shown in Table 1. A similar time course study of spleen cell subsets was done in the control group of irradiated mice with CFA and pertussis toxin. Spleens were removed and analyzed separately at every timepoint from four similarly treated mice in each group. Spleens were pressed through a stainless steel mesh and suspended in 15 ml Hanks' balanced salt solution (HBSS). Single-cell suspension was then washed twice in HBSS and centrifuged in Lympholyte-M (Cedarlane Laboratories limited, R.R.2 Hornby, Ontario, Canada) density gradient. The banded cells were washed twice in HBSS, stained with 0.5% Trypan blue and viable mononuclear cells counted.

Total of 2.0×10^5 spleen cells were incubated for 45 min at 4°C with L3T4 (PE), Lyt.2 (FITC), or MAC-1 mAb diluted 1/100 in phosphate-buffered saline (PBS) supplemented with 1% inactivated normal mouse serum (dilution buffer). The cells incubated with mAb specific to MAC-1 were washed with dilution buffer and further incubated with the secondary antibody (1/100 dilution of goat anti-rat FITC). The stained cells were then washed twice in the dilution buffer and resuspended in 500 μl PBS or fixed in 1.0% formaldehyde in PBS before flow cytometry analysis.

Cytofluorometric analyses were done using FACScan (Becton Dickinson, Mountain View, CA) flow cytometer. Lymphocytes were gated by light scatter parameters before defining subset percentages on which the calculations of absolute cell numbers were based. Median values of the four spleen cell preparations from each timepoint were used for further analysis.

2.7. Neuropathology

BALB/c EAE mice with and without SFV-A7 and SFV-A7-infected BALB/c mice were prepared for histological studies parallel to spleen cell subset analysis. Frozen sections from each mouse were stained with mAbs for $CD4^+$, $CD8^+$ and MAC-1^+ cells.

Immediately after removal of spleens the mice were perfused extensively with PBS by cardiac puncture via left chamber. The brain and spinal cord were then removed, cut to sections and frozen rapidly in Tissue-Tek O.C.T. compound (Miles, Naperville, IL). Serial frozen sections from brain, cerebellum and spinal cord were cut, mounted on a poly-L-lysine (Sigma Chemical Co., St. Louis, MO)-coated glass slides and stored at −20°C until stained. For CD4, CD8 and MAC-1 immunohistochemistry, 4–6 μm frozen sections were used. The binding of primary antibody was demonstrated with avidin–biotin method by Vectastain ABC Kit (Vector laboratories Inc., Burlingame, CA) according to the instructions of the manufacturer.

2.8. Quantification of inflammatory cell subpopulations

Positively stained cells were counted by using a modification of disector principle morphometer device (Collan et al., 1992): a single microscope with constant magnification (×317) and mirror system to reflect the microscopic image at the table where standard counting frame (correlates to 0.189 mm^2 in the tissue section) is placed. Positively stained cells in cerebellum and brain stem sections stained with anti-$CD4^+$, $CD8^+$ and MAC-1^+ mAbs were counted from four similarly treated mice at preclinical, clinical and recovered phase of the disease. Quantification of different mononuclear cell subpopulations was performed by counting 10 adjacent counting frames in cerebellum and brain stem. Counting was started randomly from different sites of frozen sections excluding damaged tissue sections, only intensively stained cells with nuclei were counted. The counted cell numbers represented 1.89-mm^2 area in

Table 1
Total number of cells and inflammatory cell subpopulations in spleens of mice treated according to different protocols

Days after induction	Cell number ($\times 10^6$)															
	CD4+				CD8+				MAC-1+				Total number of cells			
	IR	SFV [a]	EAE	EAE + SFV	IR	SFV [a]	EAE	EAE + SFV	IR	SFV [a]	EAE	EAE + SFV	IR	SFV [a]	EAE	EAE + SFV
−2 [b]	14.1	14.1	14.1	14.1	8.79	8.79	8.79	8.79	2.64	2.64	2.64	2.64	55.2	55.2	55.2	55.2
0 [c]	0.33	ND [a]	0.33	0.33	0.04	ND [a]	0.04	0.04	0.08	ND [a]	0.08	0.08	0.91	ND [a]	0.91	0.91
11	1.09	3.64 [a]	6.81	0.26	0.45	3.15 [a]	0.77	0.08	2.35	3.93 [a]	2.78	0.28	30.5	47.2 [a]	26.1	3.4
15	1.41	6.06 [a]	2.14	0.67	0.9	3.78 [a]	0.68	0.17	1.7	2.44 [a]	12.02	0.84	33.3	70.2 [a]	53.6	6.1
27	4.6	12.15 [a]	5.15	5.39	2.14	5.33 [a]	1.57	1.45	2.02	26.51 [a]	3.33	5.71	29.8	82.4 [a]	26.4	40.5

IR, group of 3.5-Gy irradiated mice; SFV, group of SFV-A7-infected mice; EAE, group of irradiated and EAE-induced mice; EAE + SFV, group of irradiated, EAE-induced mice with SFV-A7 infection.

[a] Samples are analysed at days 4, 8 and 20 after infection referring to corresponding timepoints 11, 15 and 27 days after induction of EAE.

[b] Day −2 indicates to normal control BALB/c mice 2 days before irradiation.

[c] Day 0 refers to time of EAE induction.

ND, not done. Results are median values of four similarly treated mice, except in EAE and EAE + SFV groups, the results are an average of the median value of two separate studies totalling eight mice at each timepoint.

tissue sections. The final results are given as cells per mm^2.

3. Results

3.1. Clinical observations

We have earlier shown that SFV-A7 infection increases the percentage of BALB/c mice showing clinical signs after EAE induction and shortens the mean time required for EAE development from 24 to 14 days after induction (Wu et al., 1988). The model including irradiation as the first step has been reproducible in our laboratory and control protocols with SFV-A7 infection of naive and irradiated mice or EAE induction of non-irradiated BALB/c mice with or without SFV-A7 infection did not cause clinical signs of disease during the 27-day observation period. On the other hand, irradiation and subsequent EAE induction lead to the development of paralysis and paresis in 7/20 (35%) of mice on the average of 20 days after induction. When similarly treated mice were infected with SFV-A7 virus, the proportion of paralysed mice increased to 8/9 (89%) and the clinical signs appeared on the average of 15 days after induction. It was also shown that the increase of the clinical disease was due to early development of demyelinating lesions, indicating an enhancing effect of SFV-A7 infection on the development of the autoimmune process in this model combining autoimmunity and virus infection.

3.2. Immunological cells in mouse spleen after different treatment protocols

The effect of different experimental protocols on the immunological cells in the spleens were studied by flow cytometry analysis. The purpose of the experiments was to reveal if significant systemic changes are induced by irradiation or SFV-A7 infection which may explain the enhancing effect of these treatments on EAE development. Flow cytometry analysis of spleen cells was done in groups of BALB/c mice after irradiation, after virus infection, and after EAE induction. Further, the combinations of irradiation with virus infection, irradiation with EAE induction, irradiation with EAE induction and virus infection and irradiation with CFA and pertussis toxin were also studied.

Whole body irradiation with a 3.5-Gy dose remarkably decreased the total number of splenocytes (Table 1) but lead to an increase in $CD4^+/CD8^+$ ratio 2 days after irradiation. The ratio returned to normal within 2 weeks after irradiation (Fig. 1). Intraperitoneal infection of naive and irradiated mice with SFV-A7 did not significantly affect the $CD4^+/CD8^+$ ratio (Fig. 1). Virus infection of irradiated mice results in a similar decrease in the total number of splenocytes as irradiation alone but the $CD4^+/CD8^+$ ratio remained increased until day 29 after irradiation.

EAE induction 2 days after irradiation resulted in subsequent increase of $CD4^+/CD8^+$ ratio until the administration of SFV-A7 and/or the appearance of lymphocytes in the CNS (Fig. 1). Irradiation caused the

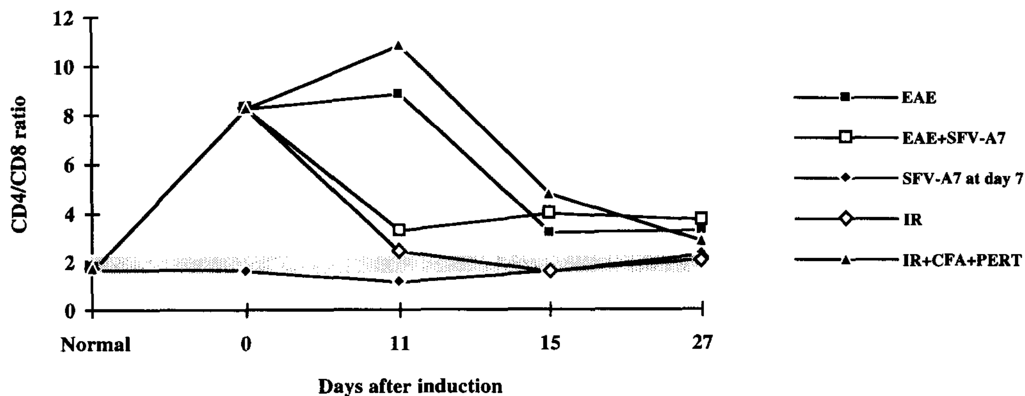

Fig. 1. Time course of $CD4^+/CD8^+$ lymphocyte ratios in spleens of mice treated according to different protocols. Irradiation primarily increases the $CD4^+/CD8^+$ lymphocyte ratio and further increase is observed in EAE-induced mice until the development of clinical signs and/or appearance of inflammatory cells into CNS. In the group with EAE induction without neuroantigen, changes in the CD4/CD8 ratio are similar to the EAE group. SFV-A7 infection does not alter the $CD4^+/CD8^+$ lymphocyte ratio in untreated mice but causes a decline in EAE-induced irradiated mice. IR, group of 3.5-Gy irradiated mice; SFV, group of SFV-A7-infected mice; EAE, group of irradiated and EAE-induced mice; EAE + SFV, group of irradiated, EAE-induced mice with SFV-A7 infection. IR + CFA + PERT, group of irradiated mice with subcutaneous CFA and subsequent pertussis toxin injections (= EAE induction without neuroantigen). Results are median values of four similarly treated and separately analysed mice, except in EAE and EAE + SFV groups, the results are an average of the median values of two separate studies totalling eight mice at each timepoint. The shaded area illustrates the range of normal values (1.4–2.2, median value 1.6) for the CD4/CD8 ratio in four healthy control mice.

initial increase of $CD4^+/CD8^+$ ratio to 8.3 which after EAE induction remained elevated; 8.3 at day 7 (data not shown) and 8.8 at day 11 after the induction. The $CD4^+/CD8^+$ ratio returned close to normal at the time when lymphocytes appeared in the CNS which occurred in EAE with virus infection on day 11 and in EAE without virus infection on day 15 (Fig. 1, Table 2). In the irradiated control mice with CFA and pertussis toxin, similar changes in CD4/CD8 ratio as in EAE-induced mice were observed but the mice remained clinically healthy (Fig. 1).

In genetically susceptible SJL mice, EAE induction resulted in an increase of the number of both $CD4^+$ and $CD8^+$ cell subpopulations and consequently in the total number of lymphocytes already before the onset of the disease 4 days after induction. The total number of mononuclear cells and $CD4^+/CD8^+$ T cell ratio decreased in the clinical phase of disease and reached the normal level in the recovery 28 days after induction.

3.3. Immunological cells in mouse brains after different treatment protocols

Although the autoimmunity is initiated in the periphery of mice in this model, the damaging effect occurs locally in the brain. Therefore, the presence of T cells and macrophages, which are the most important immunological cell populations in the autoimmune lesions, were studied at different time points after the initiation of the experiments with different protocols.

When the mice were infected with SFV-A7, a few scattered $CD4^+$ and $CD8^+$ cells appeared in the CNS 4 days after intraperitoneal injection. Some MAC-1^+ cells were also seen at the same time but their number was not increased as compared to non-infected mice which hade a few scattered MAC-1^+ cells throughout the brain. Thereafter, these cells rapidly accumulated and perivascular lymphocyte infiltrates were formed. The number of both $CD4^+$ and $CD8^+$ as well as MAC-1^+ cells increased. The infiltrates were most pronounced 8 days after infection and both lymphocytes and macrophages were present (Fig. 2). Twenty days after infection the majority of the remaining cells present in the CNS carried $CD8^+$ and MAC-1^+ markers. The number of macrophages exceeded the number of lymphocytes at all timepoints. Despite the virus infection and inflammation in the brain, the mice remained clinically healthy (Table 2).

When mice were irradiated and EAE-induced with the standard protocol, a small number of $CD4^+$, $CD8^+$ and a great number of MAC-1^+ cells were detected in cerebral white matter 11–15 days after induction in the preclinical phase of EAE. In the clinical phase 27 days after induction, $CD4^+$, $CD8^+$ and MAC-1^+ cells were present in perivascular infiltrates in the cerebral white matter. The number of cells in the CNS increased concurrently with the disease severity (data not shown). Both the number of lymphocytes and the number of macrophages in the CNS was lower than after virus infection (Table 2).

When mice were infected with SFV-A7 after EAE induction, moderate numbers of $CD4^+$ and $CD8^+$ cells appeared in the CNS 11 days after induction. The histological picture resembled the preclinical phase of EAE induced by the standard protocol. Further entry of $CD4^+$, $CD8^+$ and MAC-1^+ cells occurred during the clinical phase as seen in brain sections taken 15 days after EAE induction (Fig. 3). The number of infiltrating cells increased concurrently with the severity of the clinical disease similarly as in the EAE group without virus infection but the number of cells was nearly 10-fold. Later the number of all cell types gradually declined as the clinical recovery proceeded (Table 2).

4. Discussion

We have shown that irradiation needed for development of EAE in resistant BALB/c mice leads to a

Table 2
Clinical grades and inflammatory cell subpopulations in the CNS of mice treated according to different protocols

Days after induction	Cells/mm^2											
	CD4 +			CD8 +			MAC-1 +			Clinical grade		
	SFV [a]	EAE	EAE + SFV	SFV [a]	EAE	EAE + SFV	SFV [a]	EAE	EAE + SFV	SFV [a]	EAE	EAE + SFV
0	0	0	0	0	0	0	12.2	12.2	12.2	0	0	0
7	ND [a]	0	0	ND [a]	0	0	ND [a]	8.4	8.4	ND [a]	0	0
11	6.6 [a]	2.6	9.2	2.9 [a]	0.5	6.6	84.6 [a]	17.4	103.7	0 [a]	0	0
15	11.4 [a]	0.8	33.5	18.0 [a]	0.2	24.8	86.2 [a]	20.1	530.7	0 [a]	0	3
27	1.85 [a]	7.6	38.6	3.2 [a]	5.0	37.3	23.0 [a]	44.9	357.4	0 [a]	2.5	0

SFV, group of SFV-A7-infected mice; EAE, group of irradiated and EAE-induced mice; EAE + SFV, group of irradiated, EAE-induced mice with SFV-A7 infection.

[a] Samples were analysed from SFV-A7-infected mice at days 4, 8 and 20 after infection referring to corresponding timepoints 11, 15 and 27 days after induction of EAE.

Day 0 refers to normal control BALB/c mice. ND, not done. Results are median values of four similarly treated mice.

decrease in the total number of cells and increased the $CD4^+/CD8^+$ T lymphocyte ratio in spleen. This ratio increased further after EAE induction. SFV-A7 virus infection, which in our model enhances the development of EAE, did not change this ratio while the number of cells infiltrating the CNS increased. Enhanced entry of inflammatory cells into the CNS was detected also in untreated SFV-A7-infected mice 4–8

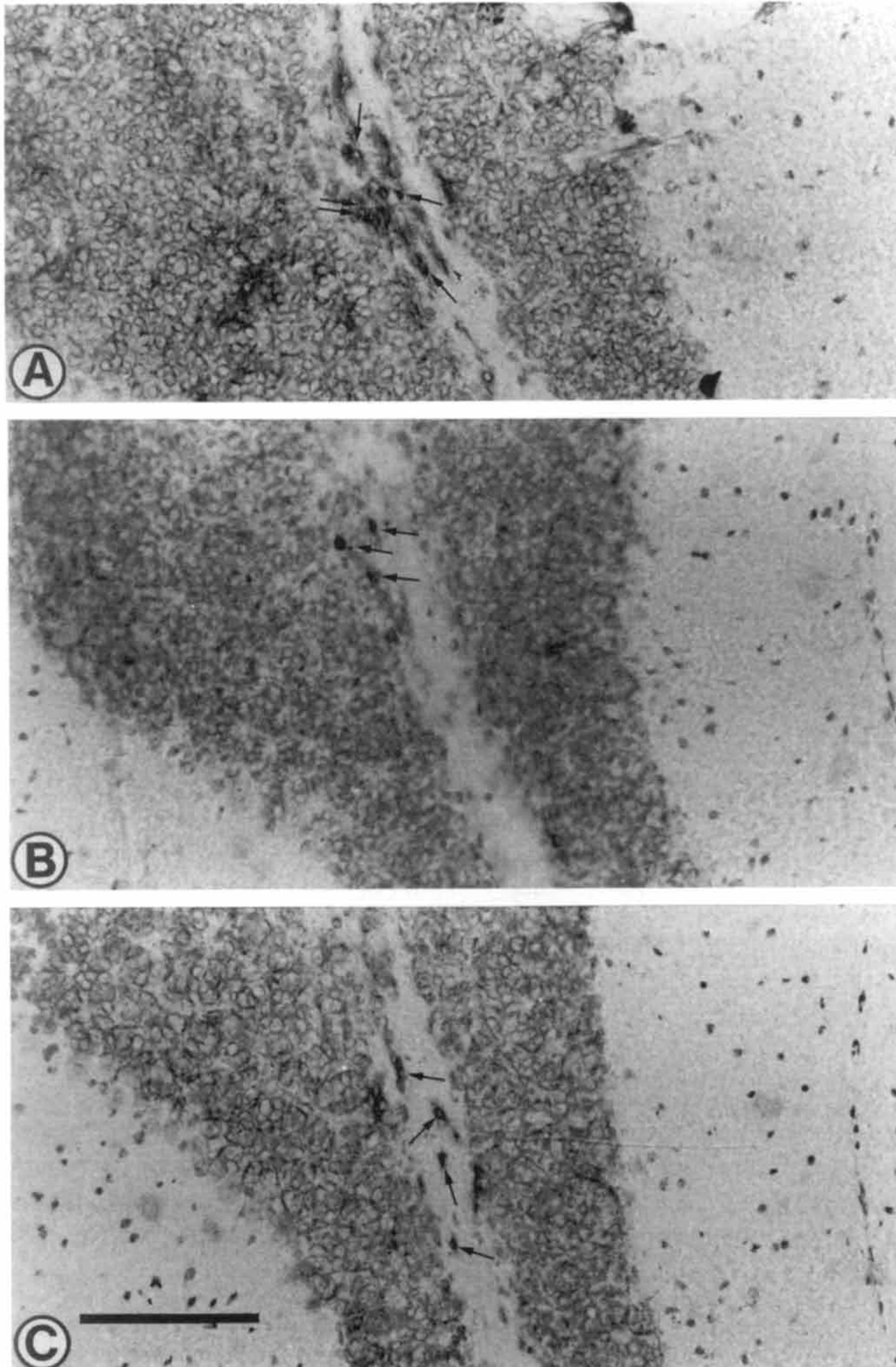

Fig. 2. Immunohistochemical detection of MAC-1^+, $CD4^+$, and $CD8^+$ cells in cerebellum of SFV-A7-infected untreated BALB/c mice 8 days after inoculation of the virus. (A) MAC-1^+ cells (arrows) in the perivascular infiltrate and in the cerebellar parenchyma. (B) $CD4^+$ (C) and $CD8^+$ lymphocytes (arrows) in serial sections representing inflammatory cell subpopulations in the same cellular infiltrate. Scale bar equals 100 μm in tissue section.

days after inoculation of the virus although there were no signs of clinical disease. Thus, active sensitization with neuroantigen is imperative for the development of autoimmune inflammation and clinical EAE.

An altered $CD4^+/CD8^+$ ratio can often be demonstrated in autoimmune diseases and is considered to be a sign of immune dysfunction although the precise consequence of this change is not known (Demaine,

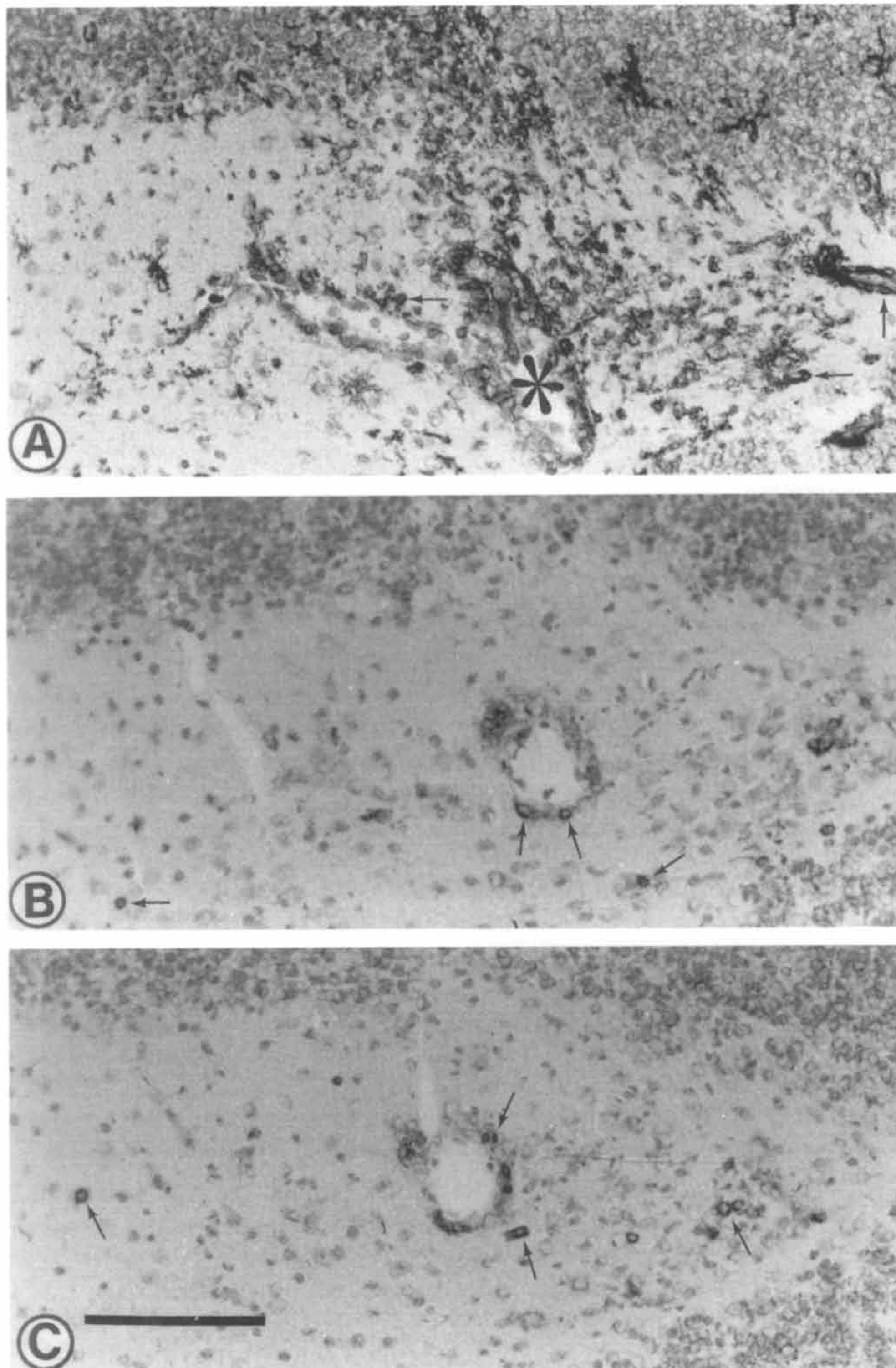

Fig. 3. Immunohistochemical detection of MAC-1^+, $CD4^+$ and $CD8^+$ cells in cerebellum of SFV-A7-infected BALB/c mice 15 days after induction of EAE (= 8 days after inoculation of the virus). (A) MAC-1^+ cells (arrows) in the perivascular infiltrate and in the cerebellar parenchyma. (B) $CD4^+$ (C) and $CD8^+$ lymphocytes (arrows) in serial sections representing inflammatory cell subpopulations in the same perivascular (blood vessel shown by an asterisk in A) cellular infiltrate. Scale bar equals 100 μm in tissue section.

1989). It can be argued that the change in the $CD4^+/CD8^+$ ratio after irradiation explains the increased sensitivity of BALB/c mice to EAE induction as the increased $CD4^+/CD8^+$ ratio was maintained until clinical disease appeared. Without EAE induction the ratio quickly returned to normal.

Irradiation is essential for the development of EAE in this model of EAE since EAE induction of non-irradiated mice does not result in the clinical disease either with or without additional infection with SFV. Irradiation is associated with general splenocyte depletion and results in the increase in $CD4^+/CD8^+$ ratio. Thus, the primary irradiation-induced increase in the $CD4^+/CD8^+$ ratio may be essential for the development of EAE. The further increase in the ratio seen after EAE induction is due to primary sensitization with neuroantigen and adjuvant and developed encephalitogenic T cells are needed for the initiation of autoimmune reactions. The increase in $CD4^+/CD8^+$ ratio after CFA and pertussis injections in irradiated mice is not enough for EAE induction because encephalitogenic cells are lacking. We consider that the change in $CD4^+/CD8^+$ ratio is of primary importance in the initiation of the autoimmune process. However, it may not be important at all after the autoimmune process has started and cells infiltrate the CNS. It has earlier been shown that EAE induction with spinal cord homogenate leads to an increased $CD4^+/CD8^+$ ratio in blood of genetically susceptible rats while the ratio remains unchanged in genetically resistant rats (Vukmanovic et al., 1990). Irradiation may thus have a stronger effect on cells which control the disease (e.g. suppressor cells) than on cells which are responsible for disease induction (antigen-specific $CD4^+$ T cells).

SFV-A7 infection of naive BALB/c mice resulted in an increase in the number of macrophages while it had no significant effect on $CD4^+/CD8^+$ cell ratios in the spleen. No further changes in $CD4^+/CD8^+$ cell ratios in spleen occurred if virus infection was given after irradiation and disease induction. It is thus unlikely that the facilitating effect on development of EAE is due to changes in immunological balance.

Infiltrating mononuclear cells are crucial for the development of EAE. It has earlier been shown that SFV-A7 infection leads to infiltration of mononuclear cells to the brain in infected mice (Berger, 1980) even if mice remain clinically healthy. Infection may also cause blood–brain barrier damage which would facilitate the entry of encephalitogenic cells and cytokines into the CNS. This is suggested by our present observation of large mononuclear cell infiltrates in EAE mice after virus infection as compared to scarce mononuclear cells in the brains of sick mice after EAE induction without virus infection. Inflammatory cells formed infiltrates consisting of $CD4^+$, $CD8^+$ and numerous $MAC\text{-}1^+$ cells. This suggests that virus infection leads to a generalized entry of mononuclear cells into the CNS in the absence of encephalitogeneic effector cells.

In EAE-induced mice, SFV-A7 infection enhances the accumulation of all inflammatory cells into the CNS compared to uninfected EAE mice. Infiltrating lymphocytes are predominantly $CD4^+$ but the proportion of $CD8^+$ cells is greater than in uninfected EAE mice. We consider that in EAE the presence of numerous activated cells in the periphery results in the increased entry of these cells into the CNS and this migration is enhanced by the virus infection. This facilitated entry may lead to increased entry of encephalitogenic cells which at this stage cannot be controlled by 'suppressor' cells. Therefore, the proportion of $CD8^+$ cells in the periphery is important in the early phase of disease but may not be important in the CNS after the autoimmune process has been initiated.

In the murine models of EAE cellular infiltrates are less extensive as compared to other rodent models of EAE. Studies on EAE in SJL mice have revealed a characteristic predominance of $CD4^+$, $CD8^+$ T cells in developing inflammatory cell infiltrates which is followed by appearance of minute B cells and macrophages shortly before onset of clinical signs of EAE. Cellular infiltrates are most pronounced in the acute phase of disease and are found perivascularily in CNS parenchyma and meninges (Sriram et al., 1982; Traugott et al., 1985). In our BALB/c model of EAE, cells with $CD4^+$ and $CD8^+$ characteristics are similarly predominant in the developing lesion and $MAC\text{-}1^+$ cells are found disseminated in CNS parenchyma. Subsequently, $MAC\text{-}1^+$ cells rapidly become the major inflammatory cell population in CNS infiltrates. The excess of $MAC\text{-}1^+$ cells in BALB/c EAE lesion in the acute phase of disease indicates the central role of phagosytosis in the demyelination process, similar to acute MS lesions.

EAE, especially the chronic form, shows many similarities to multiple sclerosis and can, therefore, be used as an animal model for MS. Clinical exacerbations in MS are often associated with virus infections (Sibley et al., 1985) and virus infections may thus trigger exacerbations. EAE in BALB/c mouse is a model of autoimmune demyelination where virus infection increases both clinical signs and neuropathological changes of the disease. This seems to be due to direct viral effects rather than changes in immune regulation. Further characterization of this model may give useful information applicable to studies on clinical relapses of multiple sclerosis.

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