Immunology 1988 **65** 23–29

Adoptively transferred experimental allergic encephalomyelitis in chimeric rats: identification of transferred cells in the lesions of the central nervous system

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Accepted for publication 18 May 1988

SUMMARY

Experimental allergic encephalomyelitis (EAE) was induced by adoptive transfer of myelin basic protein (MBP)-activated LEW spleen cells into (LEW × PVG/c) F_1→LEW chimeras. By double-immunofluorescent staining using OX27, which is specific for $RT1^c$, and monoclonal antibodies (mAb) against various T-cell antigens (TAg), inflammatory cells in the lesions of the central nervous system (CNS) were categorized into MBP-activated and transferred LEW T cells (TAg^+ $OX27^-$), accompanying T cells (TAg^+ $OX27^+$) of chimera origin and non-T cells (TAg^-OX27^+). Examination of the lesions at various stages of EAE revealed that transferred OX19 $(CD5)^+$ T cells accounted for 46% of the total number of inflammatory cells at the preclinical stage, became reduced to 23% at the clinical stage and recovered to a level between those of the preclinical and clinical stages at the recovery stage. In parenchymal infiltrates, 93% of the total T cells were transferred cells at the preclinical stage, whereas 66% were present in perivascular aggregates. At the clinical stage, the proportion of transferred T cells in the parenchyma was not different from that in the perivascular cuffs. At the recovery stage, the proportion of transferred T cells in the parenchyma was increased. Collectively, MBP-activated and transferred T cells first appeared in the CNS parenchyma followed by infiltration of T and non-T cells of recipient (chimera) origin. All these inflammatory cells formed the lesions of full-blown EAE. At the recovery stage, inflammatory cells decreased in number in all the compartments of the CNS. Transferred T cells formed the major proportion of parenchymal infiltrates at this stage. These findings strongly suggest that transferred T cells remain in the CNS parenchyma longer than cells of chimera origin and that antigen-activated T cells have well-expressed CNS-homing activity.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a T-cell-mediated autoimmune disease that is inducible in naive animals by adoptive transfer of *in vitro*-activated spleen cells from syngeneic animals immunized previously with myelin basic protein (MBP) (Matsumoto & Fujiwara, 1987) or by injection of MBP-specific T-line cells (Vandenbark, Gill & Offner, 1985). In general, it is believed that transferred cells circulate throughout the body and that some of them enter the central nervous system (CNS). These transferred cells, together with lymphocytes and macrophages of recipient origin, may form the lesions of EAE. In the previous series of studies, we attempted to elucidate the mechanism by which the histological features of EAE develop in the CNS, and found that microglia expressed major histocompatibility complex (MHC) class II antigens after infiltration of T cells into the CNS, with passively transferred EAE as well as with actively induced EAE (Matsumoto & Fujiwara, 1986, 1987; Matsumoto *et al.*, 1986; Vass *et al.*, 1986). Ia-positive microglia may thus present the antigen to the infiltrating T cells in the CNS. As to the next step, we wished to clarify the localization of the MBP-activated and transferred T cells in passive EAE.

There have been very few reports on the cell kinetics of transferred cells. Naparstek *et al.* (1983) examined the rat CNS bearing EAE by measuring the level of radioactivity present after injection of ^{57}Cr-labelled encephalitogenic T-line cells and estimated that approximately 2% of the transferred cells accumulated in the CNS. Since they did not examine the CNS autoradiographically, the proportion of transferred cells to the total amount of infiltrating inflammatory cells in the lesions of the CNS remained unknown.

In the present study, we identified transferred T cells in the lesions of EAE using bone marrow chimeras as recipients and by double-immunofluorescent staining of CNS sections with a monoclonal antibody (mAb) directed against chimera-specific MHC antigens and mAb against T-cell subsets. It was revealed

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Table 1. Monoclonal antibodies (mAb) used in the present study

mAb	Isotype	Specificity	Reference
OX19	IgG1	Lyt-1 homologue	Dallman & Thomas (1984)
W3/25	IgG1	CD4	White *et al.* (1978)
OX8	IgG1	CD8	Brideau *et al.* (1980)
OX6	IgG1	RT1.B	Williams *et al.* (1977)
OX42	IgG2a	Anti-CR3*	Robinson, White & Mason (1986)
OX27	IgG2a	RT1.A^c	Paterson *et al.*, 1987

*CR3, complement receptor type 3.

that 25–45% of the total inflammatory cells in the lesions were from the transferred cell population. Previous studies using bone marrow chimeras have provided important information on susceptibility to EAE (Singer, Moore & Williams, 1981). Moreover, unique features of adoptively transferred EAE are delineated by this approach.

MATERIALS AND METHODS

Animals

Lewis (LEW) rats were obtained from Charles River Japan Inc. (Kanagawa). PVG/c and BN rats were purchased from Siewa Sangyo (Fukuoka). Their F_1, (LEW × PVG)F_1 (LPVGF1) and (LEW × BN)F_1 (LBNF1) hybrids, were bred in the animal facilities of our laboratory. All animals were used at the age of 8–12 weeks.

Monoclonal antibodies

Monoclonal antibodies (mAb) used in the present study are listed in Table 1. All mAb, except for W3/25 and OX8 which were culture supernatants (Sera-lab, Crawley Down, Sussex, U.K.), were in the form of ascitic fluid and obtained from Serotec (Blackthorn, Bicester, Bucks, U.K.).

Preparation of radiation bone marrow chimeras

Bone marrow chimeras were prepared by irradiating LEW rats with 900 rads irradiation from a ^{60}Co source. On the same day, $7–10 \times 10^7$ bone marrow cells from LPVGF1 or LBNF1 were injected intravenously. Irradiated and reconstituted animals were maintained on autoclaved food pellets and water containing 1 mg/ml Terramycin (Taito-Pfizer Ltd, Tokyo).

Tissue sampling

Chimeric rats were killed under ether anaesthesia. The spleen, thymus and lumbar spinal cord were removed and blocks up to 1 cm^3 in size were snap-frozen in isopentane that had been precooled in a bath of acetone and dry ice. Eight-micrometer sections were cut in a cryostat, and stored at −20° until used.

Assay for chimerism

As LPVGF1 have the haplotype 1/c of MHC antigens, cells originating from transferred LPVGF1 bone marrow cells can be detected by immunohistochemical staining of chimera tissue sections using OX27 against RT1.A^c. Frozen sections of the spleen and thymus at various time intervals after bone marrow reconstitution were examined, and it was revealed that more than 95% of the mononuclear cells in the spleen had been replaced by transferred cells within 4 weeks. Therefore, in this study, all the chimeric rats were used 6 weeks after bone marrow transplantation.

EAE induction

EAE was induced by adoptive transfer of myelin basic protein (MBP)-activated spleen cells, as described elsewhere (Matsumoto & Fujiwara, 1987). Spleen cells from LEW rats previously immunized with MBP in complete Freund's adjuvant were cultured with MBP for 3 days. The culture medium consisted of RPMI-1640, 10% fetal calf serum, 1% sodium pyruvate, 1% non-essential amino acids (Gibco Lab., Life Technologies Inc., Grand Island, NY), 100 μg/ml kanamycin (Meiji Seika, Tokyo) and 5×10^{-5} M 2-mercaptoethanol. After washing, viable cells at a dose of $1–6 \times 10^7$ cells were injected i.v. into chimeric rats 6 weeks after reconstitution. In some experiments, MBP-activated LPVGF1 spleen cells were transferred into lethally irradiated LEW rats. The clinical signs of EAE were graded using the following categories: flaccid tail, grade 1; mild paraparesis, grade 2; severe paraparesis, grade 3; tetraparesis or moribund condition, grade 4.

Immunoperoxidase-staining procedures

Immunoperoxidase staining was performed essentially in the same way as described previously (Matsumoto & Fujiwara, 1986, 1987). Frozen sections were air-dried for 45 min and fixed in ether for 10 min. After incubation with normal horse serum, sections were allowed to react with mAb (ascitic fluid, 1:200; culture supernatant, 1:10), biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), 1:60 diluted with 20% normal rat serum in phosphate-buffered saline (PBS), and horseradish peroxidase (HRP)-labelled streptavidin 1:250 (Amersham International, Amersham, Bucks, U.K.). HRP-binding sites were detected in 0·05% diaminobenzidine and 0·01% hydrogen peroxide.

Double-immunofluorescent staining

All the monoclonal antibodies used in the present study were mouse IgG1 or IgG2a. Therefore, by applying fluorescent dye-labelled anti-IgG subclass antibodies as secondary antibodies, double staining in combination with IgG1 and IgG2a mAbs could be performed without producing any cross-reaction. Ether-fixed frozen sections were incubated with OX27 (IgG2a) followed by incubation with rhodamine-labelled sheep anti-mouse IgG2a (Serotec), 1:40, as the first step. Then, sections were incubated with OX19, OX8 or W3/25 (IgG1), followed by fluorescein (FITC)-labelled sheep anti-mouse IgG1 (Serotec), 1:80. Control sections were prepared by omitting the primary antibody in the first or second step. Preliminary study using OX19 (IgG1) and OX42(IgG2a) revealed that there was no cross-reaction on normal LEW spleen sections.

Quantitative analysis

Double-stained sections were observed with an Olympus BH-2 RFL epifluorescence microscope, using a ×40 objective lens. FITC-positive cells and rhodamine-positive cells in the same field were photographed individually on Fujichrome reversal film (ISO 400, Fuji Photo Film Co., Tokyo). One of the pairs of developed film was projected onto the screen of Handy Slide Projector (Cabin Co., Tokyo) and the outlines of all FITC-positive cells in the field (area, 212 × 151 μm) were traced onto

Table 2. Adoptively transferred EAE in chimeric rats*

Donor	Recipient	No. of cells transferred ($\times 10^{-7}$)	Clinical EAE	Day of onset	Max. clinical score
LEW	LEW	2	3/3	4·5	3·0†
LEW	LPVGF1→LEW	2	3/3	5	1·5
		4	6/6	4	2·4
		6	3/3	4	3·0
LEW	LBNF1→LEW	1	3/3	4	2·7
		2	4/4	4	2·9

*LEW rats were immunized with 100 μg MBP in CFA. Ten to 12 days later, spleen cells were cultured with 2 μg/ml MBP at a concentration of 2×10^6/ml for 3 days. MBP-activated spleen cells, $1–6 \times 10^7$ cells, were injected intravenously into LEW, LPVGF1→LEW, or LBNF1→LEW rats.

†The SD were within 10% of the mean values.

transparent film (OHP film, Pentel Co., Tokyo). Transparent film was then superimposed exactly over the picture on the screen on which rhodamine-positive cells were projected, and the positions of the rhodamine-positive cells were plotted. After this procedure, the T antigen (TAg) (OX19, OX8 or W3/25)-positive and OX27-positive, TAb-positive and OX27-negative, or TAb-negative and OX27-positive cells were counted separately. Statistical analysis was performed using the chi-square test.

RESULTS

Distribution of transferred LPVGF1 bone marrow cells in LPVGF1→LEW chimeras

The extent of reconstitution in the spleen, thymus and central nervous system (CNS) was examined at various time points. In the spleen and thymus 2 weeks after reconstitution, chimerism was not complete. In the spleen, there were clusters of $OX27^+$ cells, whereas in the thymus a few $OX27^+$ cells were scattered. However, 4 weeks after reconstitution, more than 95% of the mononuclear cells in the spleen expressed OX27 antigen, indicating that they were derived from transferred F_1 bone marrow cells. In the thymus, cells in the medulla were mainly replaced by LPVGF1 cells. These findings remained unchanged until 24 weeks after reconstitution. In contrast to the lymphoid organs, the CNS contained very few F_1 cells, all of which were located in the subarachnoid space (SAS) 4 weeks onwards after reconstitution. On the basis of the data obtained from this examination, chimeric rats 6 weeks after reconstitution were used for induction of EAE. In addition, the spleens and thymuses from chimeras with EAE were examined immunohistochemically using OX27, revealing that all the chimeras were in a fully chimeric state.

Clinical course of EAE in chimeras induced by adoptive transfer of MBP-activated LEW spleen cells

Various numbers of MBP-activated LEW spleen cells were injected into LPVGF1→LEW or LBNF1→LEW chimeras, and observed daily for clinical signs of EAE (Table 2). At all doses between 1×10^7 and 6×10^7, EAE was successfully transferred into chimeric rats. In most cases, chimeric rats developed clinical signs on Day 4 or 5 post-transfer (PT) followed by severe paraparesis (score 3) on Day 6 PT. All the rats recovered from EAE thereafter. On Day 10 PT, no rat showed any clinical signs. The clinical course of EAE in chimeric rats is essentially the same as that in LEW rats. When 2×10^7 sensitized cells were injected into LPVGF1→LEW chimeras, it appeared that onset was slightly delayed, and the maximal clinical signs were somewhat milder than those in other groups. We considered this finding insignificant because when sensitized LEW cells were injected into LBNF1→LEW chimeras, the latter developed full-blown EAE even at a dose of 1×10^7 cells. However, to minimize the variation in clinical severity, 4×10^7 cells were always transferred in further experiments.

Table 3. The number of inflammatory foci in LPVGF1→LEW chimeras with EAE*

Stage	Day post-transfer	No. of rats examined	Clinical score	No. of inflammatory foci
Preclinical	3–4	3	0	3·2±1·6†
Clinical	5–7	6	2·4	8·4±1·8
Recovered	8–9	3	1R‡	3·5±0·8

*Each LPVGF1→LEW chimeric rat received 4×10^7 MBP-activated LEW spleen cells.

†Sections from three different segments of the lumbar spinal cord were stained with H&E, and all inflammatory foci were counted.

‡R, recovered.

Immunopathology of EAE in chimeric rats

Immunopathological examination of the CNS from LPVGF1→LEW chimeras was done at three different stages of EAE, i.e. the preclinical, clinical and recovery stages. As shown in Table 3, histological severity was correlated well with clinical severity. At the preclinical stage, inflammatory foci were mainly located in the SAS and white matter of the spinal cord. In chimeras with clinical EAE, inflammatory foci were increased in number and located in both the gray and white matter. Immunohistochemical examination done at the clinical stage revealed that inflammatory foci mainly consisted of $OX19^+$ ($CD5^+$) and $W3/25^+$ ($CD4^+$) T cells and macrophages (Fig. 1a and b). $OX8^+$ ($CD8^+$) T cells were scarcely seen (Fig. 1c). As about 80–90% of inflammatory cells in this field were stained positively by OX27 (Fig. 1d), most cells appear to have been of chimera origin. At the recovery stage, the number of inflammatory foci was decreased in all the compartments of the CNS. $OX8^+$ T cells were still scarce at this stage. Histological findings seen in chimeras with EAE induced by adoptive transfer of sensitized LEW cells were essentially the same as those seen in LEW rats with EAE induced by adoptive transfer of LEW cells (Matsumoto & Fujiwara, 1987).

Double-immunofluorescent staining

In order to determine whether inflammatory cells were transferred LEW cells or cells from reconstituted LPVGF1 cells,

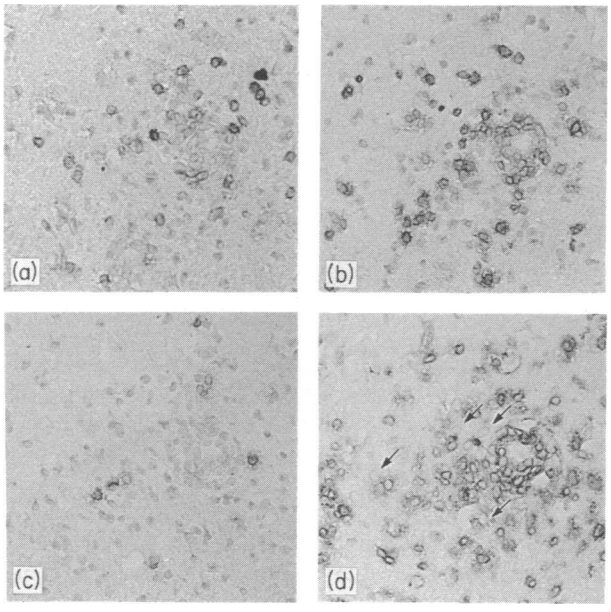

Figure 1. Immunoperoxidase staining of an inflammatory lesion of EAE. Serial frozen sections from a chimeric rat with clinical EAE were stained with OX19 (a), W3/25 (b), OX8 (c) and OX27 (d). In the lesion, a number of $OX19^+$ cells (a) and $W3/25^+$ cells (b) are visible, whereas $OX8^+$ cells (c) are few in number. About 80–90% of inflammatory cells are stained positively for OX27 (d), suggesting that they are of recipient (chimera) origin. Some $OX27^-$ cells (arrows) are also seen (× 152).

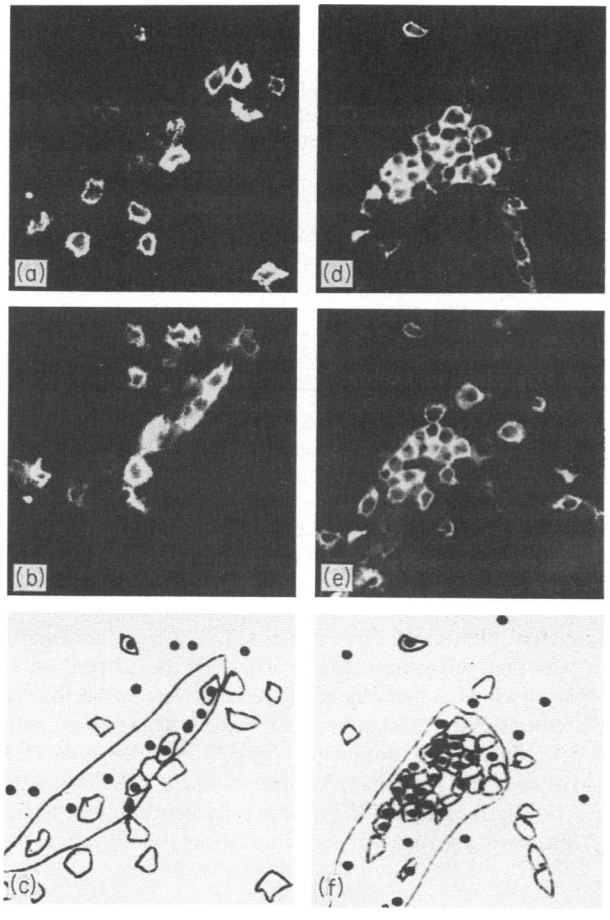

Figure 2. Double immunofluorescent staining with OX19 (a, d) and OX27 (b, e). Both $OX19^+$ cells (a) and $OX27^+$ cells (b) are located in the perivascular cell aggregate and in the parenchyma. After tracing $OX19^+$ cells and plotting $OX27^+$ cells on the same film (c), it is revealed that the majority of T cells in the parenchyma are $OX19^+OX27^-$ cells, i.e. transferred cells. $OX19^+OX27^+$ cells (cells with a solid circle) are located in the perivascular cell aggregate. (a), (b) and (c) are taken from a rat at the preclinical state (× 367). Similar findings were obtained in a rat at the clinical stage, (d), (e) and (f) (× 305).

double-immunofluorescent staining using mAb against various T-cell subsets and OX27 was performed. If certain T cells were stained positively by OX27, they would be of chimera origin. On the other hand, $OX27^-$ cells would be transferred cells. As shown in Fig. 2c and f $OX19^+$ $OX27^+$ (indicated by cells bearing a solid circle), $OX19^+$ $OX27^-$ (cells without a circle), and $OX19^-$ $OX27^+$ (solid circle) cells could be identified. Then, by the methods for quantification described earlier, the TAg (OX19, OX8 or W3/25)-positive and OX27-positive, the TAg-positive and OX27-negative, or the TAg-negative and OX27-positive cells were counted, and the percentages of each population to the total number of positive cells (TAg^+ $OX27^+$ plus TAg^+OX27^- plus TAb^- $OX27^+$) were calculated. As shown in Table 4a, $OX19^+$ $OX27^-$ cells that were $OX19^+$ cells in the transferred cell population accounted for $45{\cdot}8 \pm 4{\cdot}5\%$ of the total number of inflammatory cells at the preclinical stage. $OX19^+$ $OX27^+$ cells that were $OX19^+$ cells from the bone marrow cells of chimeras accounted for $11{\cdot}3 \pm 0{\cdot}1\%$ of the total infiltrating cells. These findings indicated that at the preclinical stage, approximately 60% of the total number of infiltrating cells were composed of T cells, most of which were transferred T cells. $OX19^-$ $OX27^+$ cells, possibly representing non-T cells (mainly macrophages), accounted for $42{\cdot}9 \pm 4{\cdot}5\%$ of the total inflammatory cells. At the clinical stage (Table 4a), the proportion of $OX19^-$ $OX27^+$ cells was increased ($59{\cdot}2 \pm 12{\cdot}3\%$), especially in the late period of this stage (data not shown). The ratio of transferred $OX19^+$ cells/total cells was decreased, indicating that the numbers of T cells and non-T cells of chimera origin were increased in the lesions. The proportion of T cells in the lesions at the recovery stage was again increased.

When the lesions of EAE were examined by W3/25 (Table 4b), the percentage of transferred $W3/25^+$ cells was very similar to that of $OX19^+$ cells at the preclinical, clinical or recovery stage. Only one minor difference was that the percentage of $W3/25^+$ cells at the clinical stage was slightly greater than that of $OX19^+$ cells. As W3/25 reacts not only with $CD4^+$ T cells but also with some macrophage (Barclay, 1981) and microglia (Matsumoto *et al.*, 1986), a certain proportion of increased positive cells compared with $OX19^+$ cells, if not all, may be attributed to macrophages and microglia.

$OX8^+$ cells in the lesions were much scarcer than $OX19^+$ or $W3/25^+$ cells. Since inflammatory foci did not always contain $OX8^+$ cells, the lesions containing $OX8^+$ cells were selected for analysis. Even under such conditions they accounted for 12–25% of the total number of stained cells (Table 4c). The most interesting finding was that some (41–45%) of $OX8^+$ cells were from the transferred cell population.

MBP-activated and transferred cells infiltrate deeply into the CNS parenchyma

In the process of quantitatively analysing the results of double immunofluorescent staining, we noticed that there was a

Table 4. Proportions of transferred T cells in infiltrates in chimeras with EAE

		$OX19^+OX27^+$*	$OX19^+OX27^-$	$OX19^-OX27^+$
(a)				
Preclinical	(24)‡	11·3±0·1‡	45·8±4·5	42·9± 4·5
Clinical	(43)	17·5±6·6	23·1±6·2	59·2±12·3
Recovered	(22)	17·0±5·5	37·4±5·4	45·6±10·3
(b)		$W3/25^+OX27^+$	$W3/25^+OX27^-$	$W3/25^-OX27^+$
Preclinical	(15)	14·2±3·0	43·6±1·5	42·1± 1·4
Clinical	(28)	22·8±5·5	32·3±4·5	44·9± 5·0
Recovered	(16)	20·1±5·7	37·0±5·7	42·9± 1·2
(c)		$OX8^+OX27^+$	$OX8^+OX27^-$	$OX8^-OX27^+$
Preclinical	(15)	9·0±1·5	7·4±0·8	83·7± 2·3
Clinical	(27)	11·7±4·6	8·1±3·1	80·2± 5·4
Recovered	(14)	14·2±1·9	10·6±4·3	75·2± 2·4

*The function and origin of the three subpopulations of inflammatory cells are as follows: $OX19^+$ $OX27^+$ cells are T cells of chimera origin; $OX19^+$ $OX27^-$ cells are transferred T cells; and $OX19^-$ $OX27^+$ cells are non-T cells of chimera origin.

†Number of fields examined.

‡A given cell population was counted, and the percentage of the cell population to total stained cells (TAg^+OX27^+ plus TAg^+OX27^- plus TAg^-OX27^+) was calculated.

tendency for $OX27^-$ T cells, i.e. transferred cells, to be located in the CNS parenchyma, whereas $OX27^+$ T cells, which are of chimera origin, were found in perivascular inflammatory cell aggregates (Fig. 2). Therefore, we plotted and counted TAg^+ (OX19, W3/25 or OX8) and $OX27^+$, or the TAg^+ and $OX27^-$ cells inside or outside the margin. As shown in Table 5, 93% of $OX19^+$ T cells in the parenchyma were $OX27^-$ T cells, whereas 6·9% were $OX27^+$ T cells at the preclinical stage. In perivascular cells aggregates, 34% of $OX19^+$ T cells were $OX27^+$ cells. At the clinical stage of EAE, the percentage of $OX19^+$ $OX27^-$ T cells in both the perivascular cuffings and parenchyma was decreased. In three of five cases examined, there was no difference in the composition of T cells ($OX27^+$ vs. $OX27^-$) either inside or outside the cell aggregates. These findings suggested that at the preclinical stage, large numbers of inflammatory cells in the lesions were T cells and that 80% of T cells (93% in the parenchyma) were from the transferred population. At the clinical stage, predominance of $OX19^+$ $OX27^-$ cells in the parenchyma became less obvious. This was probably because T cells of chimera origin appeared later in the CNS and became distributed in both perivascular cuffings and the parenchyma. At the recovery stage, $OX27^-$ T cells were predominant over $OX27^+$ T cells in the parenchyma, whilst in the perivascular cell aggregates the composition remained unchanged.

Table 5. Proportions of transferred T cells in perivascular aggregates and in parenchymal infiltrates

		Perivascular aggregates*		Parenchymal infiltrates	
		$OX19^+$ $OX27^+$	$OX19^+$ $OX27^-$	$OX19^+$ $OX27^+$	$OX19^+$ $OX27^-$
Preclinical	(3)†	33·7± 1·7	66·3± 1·7	6·9±0·6	93·1±0·5‡
Clinical	(5)	55·7±19·7	44·3±19·7	33·0±7·6	67·0±7·6§
Recovered	(3)	68·7±14·6	33·3±14·6	15·4±4·3	84·6±4·3¶

*Including inflammatory cells in the subarachnoid space.

†The number of cases examined.

‡The percentage of $OX19^+OX27^-$ cells in parenchymal infiltrates was compared with that in perivascular cell aggregates in each case using the chi-square test. The proportion of $OX19^+OX27^-$ cells in parenchymal infiltrates was significantly greater than that in perivascular aggregates in all cases ($P<0·005$).

§Insignificant in three cases out of five.

¶Significantly greater in all cases ($P<0·005$).

With regard to $W3/25^+$ cells, the same tendency was observed as that for $OX19^+$ cells (data not shown). However, $OX8^+$ cells observed in the lesions were too scarce to analyse in this manner.

EAE induced in irradiated LEW recipients by transfer of MBP-activated LPVGF1 spleen cells

In the study using chimeras as recipients, transferred cells were identified by nature of the fact that a given cell was negative for OX27. If surviving LEW cells after receiving a lethal dose of irradiation (less than 5% in the spleen) were recruited in the CNS lesions, this population would not be distinguished from transferred cells. In order to exclude the possibility that surviving LEW cells might contribute to the lesion formation, MBP-activated LPVGF1 spleen cells were transferred into irradiated LEW rats, and the number of transferred cells in the lesions was compared to that in the lesions of chimeras. When F_1 spleen cells were transferred into non-irradiated or 700-rads irradiated LEW rats, the recipients did not develop EAE. After 1000-rads irradiation, however, EAE was successfully transferred into LEW rats. Histological examination of the CNS in rats with clinical EAE revealed that in contrast to EAE in chimeras, there was a relatively small number of inflammatory cells. Perivascular inflammatory cell aggregates were small and few in number. This was probably because there were very few accompanying cells of recipient origin due to the lethal dose of irradiation given to the recipients. However, in each lesions of EAE, $OX27^+$ $OX19^+$ (indicating transferred cells in this system), $OX27^+$ $OX8^+$ or $OX27^+$ $W3/25^+$ T cells were recognized. Furthermore, transferred cells in an area of 212×151 μm in the lesions of irradiated LEW were counted and compared with those seen in the lesions of chimeras. The results revealed that the number of transferred cells in irradiated recipients (9·6±7·1) was insignificantly different from that in chimeric rats (12·6±5·2). These findings strongly suggested that surviving LEW cells after irradiation may not contribute to the lesion formation and that when MBP-activated spleen cells are transferred, both $W3/25^+$ and $OX8^+$ transferred T cells accumulate in the CNS of recipients.

DISCUSSION

There are several autoimmune diseases characterized by organ-specific inflammation elicited by imunization of organ-specific antigens. In the case of EAE, inflammation is elicited by

immunization of brain-specific antigens, myelin basic protein or proteolipid apoprotein (Endoh *et al.*, 1986; Satoh *et al.*, 1987; Yamamura *et al.*, 1986) and is localized in the CNS. As well as EAE, experimental autoimmune neuritis (Izumo *et al.*, 1985; Linington *et al.*, 1984), uveoretinitis (Capsi *et al.*, 1986; Rozenszajn *et al.*, 1986), thyroiditis (Maron *et al.*, 1983; Romball & Weigle, 1987), arthritis (Cohen *et al.*, 1985; Englert *et al.*, 1986; Holoshitz *et al.*, 1983; Van Eden *et al.*, 1985), interstitial nephritis (Mann *et al.*, 1985; Zakheim *et al.*, 1984) and orchitis (Tung *et al.*, 1987) are all categorized within this disease entity. Organ-specific autoimmune diseases are also inducible by adoptive transfer of sensitized spleen or antigen-specific T-line cells. Analysis of T-cell lines and clones has revealed that these diseases are mediated by $CD4^+$ T cells (Holshitz *et al.*, 1983; Linington *et al.*, 1984; Romball & Weigh, 1987; Rozenszajn *et al.*, 1986; Zamvil *et al.*, 1985). However, as far as we are aware, there has been no report concerning the localization of transferred T cells in the lesions.

In the present study, we were able to identify transferred T cells using chimeras as recipients. It was revealed that percentage ratios of transferred $OX19^+$ T cells to the total number of inflammatory cells were 45·8% at the preclinical stage, 23% at the clinical stage, and 37·4% at the recovery stage. Analysis of the relative proportions of infiltrating cells in perivascular cell aggregates and in parenchymal infiltrates revealed several interesting findings. In perivascular cell aggregates, the proportion of transferred T ($OX19^+$ $OX27^-$) cells was highest at the preclinical stage, and became reduced thereafter, showing a range between 33·3% and 66·3%. In parenchymal infiltrates, however, transferred T cells accounted for 93·1% of the total number of T cells at the preclinical stage. The percentage showed a reduction at the clinical stage, but rose to almost the previous level again later. Comparison of transferred T cells in parenchymal infiltrates with those in perivascular aggregates revealed that the percentage of transferred T cells in the former was significantly greater than that in the latter in all cases examined at the preclinical stage. In contrast, at the clinical stage, no significant difference was observed in some cases. In other words, both transferred T cells and T cells of chimera origin were distributed equally inside and outside the perivascular aggregates. At the recovery stage, the percentage of transferred T cells in the parenchyma again increased.

For adoptive transfer of EAE, we used spleen cells from MBP-immunized rats after short culture with MBP. Since they were a mixture of MBP-reactive T cells and bystander cells, transferred T cells identified in the lesions of the CNS were not always MBP-reactive T cells. However, it is known that encephalitogenicity of spleen cells after short culture with MBP is 10 times greater than that before culture (Richert *et al.*, 1979; Smith & Waksman, 1969). Therefore, transferred T cells found in the CNS appear to contain the certain number of MBP-reactive T cells.

Figure 3 illustrates the findings obtained in the present study. At the preclinical stage, infiltrating inflammatory cells were located mainly in the SAS and Virchow–Robin space and a few were located in the parenchyma. Inflammatory cells in the SAS consisted of MBP-activated and transferred T cells, accompanying T cells and macrophages of chimera origin. In the parenchyma, most infiltrating cells were transferred T cells. At the clinical stage, macrophages and T cells of chimera origin rapidly entered the CNS parenchyma. The cell composition of the parenchymal infiltrates then became almost the same as that in perivascular aggregates. At the recovery stage, inflammatory cells in all compartments decreased in number, and the percentage of transferred T cells in the parenchyma again increased. Taken together, MBP-activated and transferred T cells appeared first in the CNS parenchyma and existed for a longer period than macrophages and T cells of chimera origin.

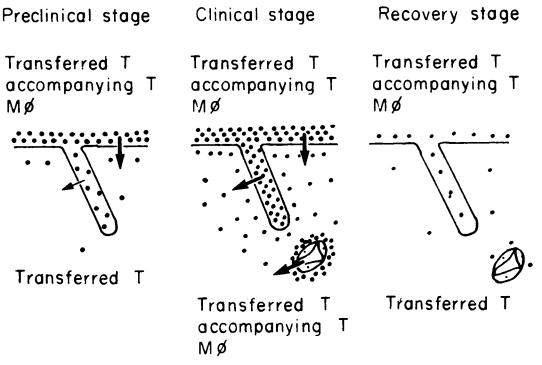

Figure 3. Schematic representation of histology of EAE induced by adoptive transfer of MBP-activated LEW spleen cells into LPVGF1→LEW chimeras. At the preclinical stage, inflammatory cells are mainly located in the subarachoid space (SAS) and Virchow–Robin space and a few are located in the parenchyma. Inflammatory cells in the SAS are composed of MBP-activated and transferred T cells, accompanying T cells and macrophages of recipient (chimera) origin. In contrast, a large number of infiltrating cells in the parenchyma are transferred T cells. At the clinical stage, macrophages and T cells of recipient origin rapidly enter the CNS parenchyma. Thus, the cell distribution seen at the preclinical stage becomes less obvious. At the recovery stage, inflammatory cells in all the compartments become decreased in number. However, most T cells seen in the parenchyma are transferred T cells.

In the present study, we identified transferred cells in the lesions of passive EAE. Since transferred T cells had a tendency to become localized in the CNS parenchyma, it was strongly suggested that transferred T cells play a major role in the development of EAE.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Neuroimmunological Research Committee, Ministry of Health and Welfare, Japan.

We thank M. Watanabe for secretarial assistance, and M. Kenmotsu and T. Hashimoto for technical assistance.

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