

TRANSFER OF EXPERIMENTAL ALLERGIC  
ENCEPHALOMYELITIS TO BONE MARROW CHIMERAS  
Endothelial Cells Are Not a Restricting Element

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Ia<sup>+</sup> cells of non-bone marrow origin (e.g., endothelial cells, astrocytes) have been proposed to play a significant and perhaps determining role in the expression of a cell-mediated response to neuroantigens (1-4). To test this premise in vivo we prepared F1-to-parent bone marrow chimeras and used these chimeras as recipients in the adoptive transfer of experimental allergic encephalomyelitis (EAE). Our results indicate that the development of EAE within the recipient is dependent upon MHC compatibility between adoptively transferred lymphocytes and bone marrow-derived cells. MHC compatibility between transferred lymphocytes and non-bone marrow-derived cells was not a requirement for adoptive transfer of clinical disease.

**Materials and Methods**

**Animals.** Inbred Lewis [LEW (RT-1<sup>l</sup>)], Brown Norway [BN (RT-1<sup>n</sup>)], Fischer-344 [F-344 (RT-1<sup>bu</sup>)], Buffalo [BUF (RT-1<sup>b</sup>)], and ACI (RT-1<sup>a</sup>) rat strains were obtained from Microbiological Associates, Walkersville, MD, and from Simonson Laboratories, Inc., Gilroy, CA. The following (LEW × Parent-2)F<sub>1</sub> rat strains were bred and maintained locally: (LEW × BN)F<sub>1</sub> (RT-1<sup>ln</sup>), (LEW × BUF)F<sub>1</sub> (RT-1<sup>lb</sup>), and (LEW × ACI)F<sub>1</sub> (RT-1<sup>la</sup>). Animals were provided access to food and water without restriction and were watered by hand during periods of paralysis.

**Purification of Myelin Basic Protein.** Guinea pig brains were purchased from Pel-Freez Biologicals (Rogers, AR) and stored at -70°C until extracted for basic protein (BP). BP was prepared according to a procedure modified from Diebler et al. (5).

**Immunization with BP-CFA.** Myelin BP was rehydrated in saline at a concentration of 1 mg/ml and emulsified in an equal volume of CFA containing 10 mg/ml of nonviable, desiccated *Mycobacterium tuberculosis* H37 RA. Active immunization with BP was accomplished by injecting 0.1 ml of the BP-CFA divided equally between the two front foot pads.

**Adoptive Transfer of EAE.** The procedure was followed as described (7).

**Production of Bone Marrow Chimeric Animals.** Animals received 900-1,000 rad of irradiation using a <sup>60</sup>Cobalt source. After irradiation, hematopoietic function was restored by bone marrow reconstitution. Chimeric rats were used as recipients not sooner than 6 mo after bone marrow engraftment.

**Irradiation of Cell Recipients.** Where indicated, cell recipients received 900-1,000 rad of irradiation using a <sup>60</sup>Cobalt source 24 h before cell transfer.

**Clinical Evaluation.** Rats used in adoptive transfer experiments were evaluated daily for clinical signs of neurologic impairment and graded on a scale of 1-3: animals with

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TABLE I  
MHC Restriction of Adoptively Transferred EAE

Donor*	Recipient* <sup>‡</sup>	Nonirradiated		Irradiated <sup>†</sup>	
		Disease/total <sup>†</sup>	Symptoms**	Disease/total <sup>†</sup>	Symptoms**
LEW	LEW	8/8	2.7	6/6	2.8
	BN	0/4	0.0	0/4	0.0
	BUF	0/4	0.0	0/4	0.0
	F-344	0/6	0.0	6/6	2.7
	(BN × LEW)F <sub>1</sub>	6/6	2.6	5/5	2.4
	(ACI × LEW)F <sub>1</sub>	7/7	2.8	6/6	2.6
(ACI × LEW)F <sub>1</sub>	(ACI × LEW)F <sub>1</sub>	6/6	2.4	5/5	2.6
	LEW	0/7	0.0	6/6	2.7
	ACI	0/5	0.0	6/6	2.2
	BN	0/4	0.0	0/4	0.0

\* Spleen cells were obtained from donors 12–14 d after BP-CFA immunization. Cells were cultured in the presence of BP for 72 h and were subsequently transferred to recipients.

<sup>‡</sup> Recipients received  $2 \times 10^7$  cells.

<sup>†</sup> MHC haplotypes of recipient animals are as follows: LEW, RT-1<sup>l</sup>; F-344, RT-1<sup>bl</sup>; BN, RT-1<sup>n</sup>; BUF, RT-1<sup>b</sup>.

<sup>†</sup> Irradiated recipients received 900–1,000 rad of irradiation 24 h before cell transfer.

<sup>†</sup> Number of animals with clinical signs of disease per total animals per group; clinical disease indices are described in Materials and Methods.

\*\* Average maximum clinical signs that develop after disease onset.

flaccid tails were given a grade 1; animals with hindquarter weakness or paralysis were given a grade 2; and hindquarter paralysis with incontinence was given a grade 3.

**Histology.** Tissue was removed from selected recipients at the time of maximum clinical disease. After fixation in 10% buffered formaldehyde solutions, spinal cords were carefully isolated from the surrounding tissue. The cords were subdivided into three segments and embedded in paraffin. At least eight sections were cut from each block and stained with hematoxylin and eosin.

## Results and Discussion

The adoptive transfer of clinical EAE is readily achieved with spleen cells obtained from BP-sensitized donors if these cells are stimulated in vitro with specific antigen before transfer. Table I shows the development of adoptively transferred clinical EAE in syngeneic and semisyngeneic, but not allogeneic recipients of BP-stimulated spleen cells obtained from BP-CFA-immunized Lewis (LEW) and (ACI × LEW)F<sub>1</sub> rats. These results are consistent with other reports of MHC restriction in the transfer of EAE in the rat (6, 7). In addition to exhibiting a requirement for MHC compatibility, presumably reflecting the need for in vivo antigen presentation requirements, transferred cells must escape host rejection mechanisms during the inductive phase of the disease. For example, clinical EAE develops in experiments where LEW cells are transferred to F344 rats or (LEW × BN)F<sub>1</sub> cells are transferred to LEW rats only if the recipient is irradiated before transfer. While these donor-recipient combinations have shared MHC antigens, recipient recognition of foreign non-MHC antigens on the transferred cells apparently inhibits donor cell function in the nonirradiated recipient. Irradiation of recipients before transfer, however, does not allow MHC-incompatible cells to transfer EAE, indicating a requirement for proper antigen presentation within the recipient.

The restriction patterns seen in Table I do not separate the APC role of Ia<sup>+</sup>, nonhematogenous cells from that of the bone marrow-derived mononuclear cell populations in the development of adoptively transferred EAE. The data pre-

TABLE II  
*Adoptive Transfer EAE in Bone Marrow Chimeras*

Donor*	Recipient <sup>‡</sup>	Disease/total <sup>§</sup>	Symptoms <sup>¶</sup>	Dis- ease <sup>¶</sup> onset	Disease** recovery
LEW	LEW	6/6	2.6	5.2	8.1
LEW	(LEW × BN) <sub>F<sub>1</sub></sub> →BN Bone marrow chimera	11/11	2.7	5.4	8.5
LEW	BN	0/4	0.0	—	—
ACI	ACI	4/4	2.2	5.5	8.2
ACI	(ACI × LEW) <sub>F<sub>1</sub></sub> →LEW Bone marrow chimera	5/6	2.4	5.3	8.3
ACI	(LEW × BN) <sub>F<sub>1</sub></sub> →BN Bone marrow chimera	0/3	0.0	—	—

\* Spleen cells were obtained from donors 12–14 d after BP-CFA immunization. Cells were cultured in the presence of BP for 72 h and were subsequently transferred to recipients.

<sup>‡</sup> Recipients received  $2 \times 10^7$  cells.

<sup>§</sup> Number of animals with clinical signs of disease per total animals per group; clinical disease indices are described in Materials and Methods.

<sup>¶</sup> Average maximum clinical signs that develop after disease onset.

<sup>¶</sup> Average time of onset (days) of grade 2 clinical signs.

\*\* Average time (days) at which recovery from clinical disease was complete.

sented in Table II demonstrate that (LEW × BN)<sub>F<sub>1</sub></sub>→BN bone marrow chimeras developed clinical signs of disease after transfer of LEW cells similar to those seen with syngeneic transfer of BP-activated LEW spleen cells. In contrast, transfer of allogeneic (ACI) BP-activated spleen cells to these bone marrow chimeras did not result in clinical or histopathologic signs of EAE. However, (ACI × LEW)<sub>F<sub>1</sub></sub>→LEW bone marrow chimeras developed clinical disease when infused with BP-reactive ACI-derived spleen cells. In all cases, histologic disease routinely associated with clinical EAE (8) was evident in animals that had exhibited signs of clinical disease (results not shown).

The chimeras used in this study were designed to test the potential influence of non-bone marrow-derived cells on the development of adoptively transferred EAE. Endothelial cells isolated from the cerebral vasculature express Ia when isolated from SJL mice exhibiting clinical EAE, but Ia<sup>+</sup> endothelial cells are not found in similar preparations from normal syngeneic mice (1). In guinea pigs immunized with BP, endothelial cells become Ia<sup>+</sup> just before disease onset (9). However, in (Strain 2 × Strain 13)<sub>F<sub>1</sub></sub> hybrids, only the high-responder strain 13 haplotype is expressed on endothelial cells of BP-sensitized guinea pigs (10). Large numbers of Ia<sup>+</sup> cells are also found in multiple sclerosis brain lesions, especially in the peripheral areas of the expanding plaque (11). These observations have led some investigators to suggest that endothelial cells may have a relevant role in antigen presentation in vivo (9, 12), making them an active and central participant in the expression of antigen-specific delayed-type hypersensitivity responses (13) in general and in the development of the autoimmune response to antigens of the central and peripheral nervous tissue.

Our results question the need for MHC-restricted antigen presentation by endothelial cells in vivo. Table I shows that LEW donor cells transfer clinical EAE only to recipients that share the LEW MHC and that the (LEW × BN)<sub>F<sub>1</sub></sub> recipient develops a severity of clinical disease similar to that seen with syngeneic recipients. In recipients that develop disease, antigen presentation could be the responsibility of endothelial cells or astrocytes as well as bone marrow-derived

TABLE III  
*Persistence of Donor Derived BP Sensitive Cells in Bone Marrow Chimeras*

Donor*	Primary Recipient <sup>‡</sup>	Secondary Culture <sup>‡</sup>	Secondary Recipient	Disease <sup>‡</sup> /total	Symptoms <sup>‡</sup>
LEW	(LEW × BN)F <sub>1</sub> →BN	BP	LEW	4/4	2.2
	(LEW × BN)F <sub>1</sub> →BN	BP	BN	0/4	0.0
	(LEW × BN)F <sub>1</sub> →BN	—	LEW	0/4	0.0
LEW	(ACI × LEW)F <sub>1</sub> →ACI	BP	LEW	4/4	2.4
	(ACI × LEW)F <sub>1</sub> →ACI	BP	ACI	0/3	0.0
	(ACI × LEW)F <sub>1</sub> →ACI	BP	ACI <sub>irr</sub>	0/4	0.0

\* Spleen cells were obtained from donors 12–14 d after BP-CFA immunization. Cells were cultured in the presence of BP for 72 h and were subsequently transferred to recipients.

<sup>‡</sup> Recipients received  $2 \times 10^7$  cells. All were bone marrow chimera.

<sup>‡</sup> Spleen cells were obtained from primary recipients 10–21 d after recovery from the initial episode of clinical disease. The cells were cultured with BP for 72 h before transfer to secondary recipients.

<sup>‡</sup> Number of animals with clinical signs of disease per total animals per group; clinical disease indices are described in Materials and Methods.

<sup>‡</sup> Average maximum clinical signs that develop after disease onset.

cells. However, chimeric recipients of the (LEW × BN)F<sub>1</sub>→BN construct would possess non–bone marrow–derived cells allogeneic to the transferred LEW spleen cells. Since Table II shows that these chimeric recipients develop and recover from clinical disease with the same kinetics as syngeneic recipients, any MHC-restricted antigen-presenting function by these non–bone marrow–derived cells is not required for the development of adoptively transferred EAE.

We have previously shown (7) that rats that have recovered from adoptively transferred EAE can serve as a source of transfer-active cells and that this transfer-active population is derived from the original donor cell inoculum. It is evident from the data presented in Table II that recovery from adoptively transferred clinical disease in the BN chimeras is temporally consistent with recovery after syngeneic transfer of clinical disease. Furthermore, spleen cells obtained from chimeric recipients of transfer-active LEW cells can serve as a population of transfer-active cells (Table III). Notably, serial transfer of EAE by this cell population is still dependent on *in vitro* activation by spleen cells. The recipient combinations exhibiting successful serial transfer of clinical disease also suggest that BP-specific LEW cells survive in the chimeric environment and can subsequently transfer clinical disease to LEW recipients. In contrast, serial transfer to BN or ACI recipients was not successful. These latter results also support our previous observations (7) that the development of adoptively transferred EAE does not result in the recruitment of BP-reactive cells derived from the recipient's lymphoid compartment.

If the endothelium does not play an active, antigen-presenting role in the development of EAE, how then does the inflammatory lesion develop? The ability to transfer EAE to a recipient in which the endothelium is allogeneic to the transferred cells may be explained by an interaction of T cells, T cell products, and mast cells (14–17). In support of this model we (18) and others (19–22) have reported that the clinical signs of EAE can be prevented in animals that have received compounds that alter mast cell release of histamine and serotonin.

In addition to lending indirect support of the Askenase-Loveren model of DTH (17), our results would also suggest that the clinical development of EAE is not caused by the direct interaction of MHC-restricted cytotoxic cells and central nervous system (CNS) tissue. The response of chimeric recipients to the

transfer of BP-specific lymphocytes (Table II) indicates that the presence of semisyngeneic F<sub>1</sub> bone marrow-derived accessory cells are sufficient for disease induction, even though the transferred lymphocytes are allogeneic to the CNS. In these donor-recipient combinations the only possible MHC compatibility is with the transferred lymphocytes and the established bone marrow. Non-bone marrow-derived CNS tissue would not display MHC compatibility with the transferred lymphocytes. Consequently, MHC-restricted cytotoxic cells would not be expected to function in the development of clinical disease.

Our observation that the endothelium need not be MHC compatible with BP-specific lymphocytes to have these cells function in vivo may be relevant to general considerations of the role of Ia<sup>+</sup>, non-bone marrow-derived cells and the antigen-presenting requirement for delayed-type hypersensitivity responses. It is probable within the syngeneic system that all Ia<sup>+</sup> cells, independent of origin, are involved in antigen presentation at some point in the immune response. However, the results of our studies would argue that any antigen-presenting function of endothelial cells in vivo is secondary to that of bone marrow-derived cells and that the development of EAE is not influenced by the lack of antigen presentation, or of compatible Ia<sup>+</sup> expression, by cells of the endothelial barrier.

### Summary

The adoptive transfer of clinical and histopathologic signs of experimental allergic encephalomyelitis (EAE) requires MHC compatibility between cell donor and cell recipient. The results of adoptive transfer studies using F<sub>1</sub> to parent bone marrow chimeras as recipients of parental-derived BP-sensitive spleen cells indicate that this restriction is not expressed at the level of the endothelial cell but is confined to the cells of bone marrow derivation. Furthermore, these results indicate that the development of EAE is not dependent on the activity of MHC-restricted cytotoxic cells.

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