

# Insulin-Like Growth Factor-1 Fails to Enhance Central Nervous System Myelin Repair during Autoimmune Demyelination

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**Previous studies have shown that insulin-like growth factor-1 (IGF-1) has beneficial effects, both clinically and histopathologically, on experimental autoimmune encephalomyelitis (EAE), although results vary depending on species and treatment regimen. The present study investigated whether IGF-1, delivered at different time points during the acute and chronic phases of adoptively transferred EAE in SJL mice, had the ability to affect or enhance myelin regeneration. Central nervous system tissue sampled at different stages of treatment was subjected to detailed neuropathological, immunocytochemical and molecular analysis. The results revealed some transient clinical amelioration and low level remyelination after IGF-1 administration during the acute phase of EAE. However, central nervous system tissue from acute phase treated animals sampled at chronic time points and from animals given IGF-1 during the chronic phase revealed no enhancing effect on remyelination in comparison to vehicle-treated controls. Examination of oligodendrocyte progenitor populations also revealed no differences between IGF-1- and vehicle-treated groups. At the cytokine level, the immunomodulatory molecules TGF- $\beta$ 2 and TGF- $\beta$ 3 displayed significant decreases that may have contributed to the transient nature of the effect of IGF-1 on EAE. Together with evidence from previous studies, it appears doubtful that IGF-1 is a good candidate for treatment in multiple sclerosis, for which EAE serves as a major model. (*Am J Pathol* 2000, 157:933–943)**

Experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease of the central nervous system (CNS) and the prime animal model for multiple sclerosis (MS), can be induced by active or passive sensitization to myelin antigens and has been particularly useful in the testing of therapeutic compounds relevant to MS.<sup>1,2</sup> While attempts to abrogate EAE have traditionally

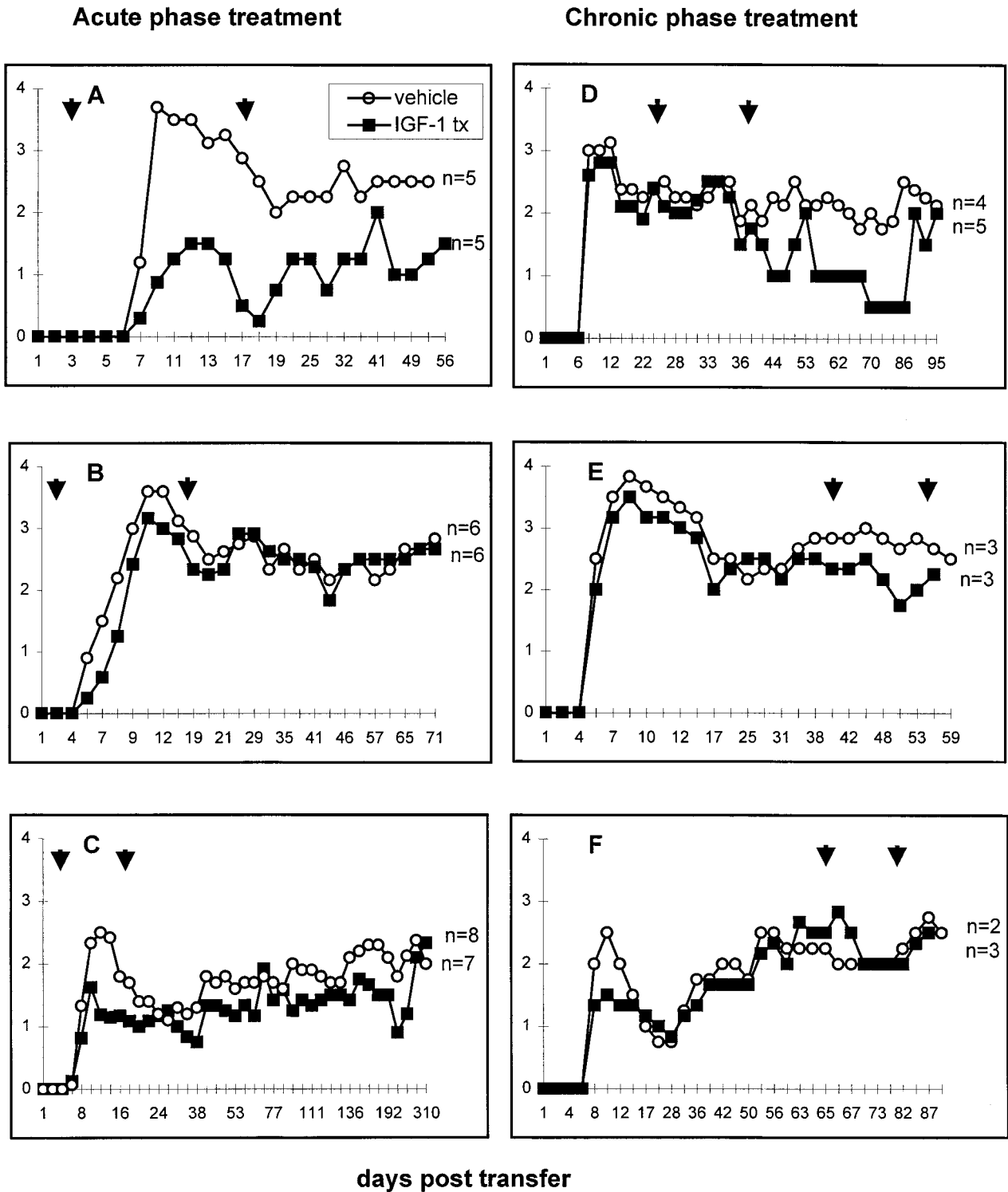
involved immunological strategies and myelin antigens, it has become apparent in recent years that a number of pathways remote from autoimmune approaches may also have therapeutic relevance. One approach currently being explored in this regard is treatment of EAE with growth factors with known regulatory effects on myelinating cells. Among these, the neuregulin glial growth factor 2 (GGF2) has been shown to have a stimulatory effect on oligodendrocytes *in vitro*.<sup>3</sup> This feature led to its being tested in EAE where it was found to have a beneficial effect on the clinical and pathological outcome of disease, an effect corroborated by the observation of enhanced myelin repair, myelin gene up-regulation and suggestions of a switch from a proinflammatory to an immunoregulatory cytokine profile.<sup>4</sup>

Another growth factor, insulin-like growth factor-1 (IGF-1), has been shown like GGF2, to promote oligodendrocyte development and stimulate myelin gene expression *in vitro*.<sup>5,6</sup> Previous studies *in vivo* have shown that IGF-1 is capable of reducing clinical signs and lesion severity in the Lewis rat and SJL mouse models of acute and chronic relapsing EAE.<sup>7,8</sup> Another laboratory has shown somewhat different results in the (PL  $\times$  SJL/J) F1 mouse model of EAE,<sup>9</sup> where a delay in disease onset was seen after treatment during the acute phase. However, once signs developed, the disease was more severe with higher doses of IGF-1, and treatment of chronic animals had no effect. Since CNS remyelination is a major ultimate goal of many therapeutic protocols in MS, the present study was designed to compare the long-term effects of IGF-1 delivered during both the acute and chronic phases of chronic relapsing EAE from the standpoint of myelin gene expression and repair. The findings have shown some transient clinical improvement and low-level remyelination during the acute phase, but no clinical improvement and no difference in the level of remyelination between controls and groups chronically

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**Figure 1.** Clinical charts of three experiments showing mice treated during the acute phase of EAE (A-C) and during the chronic phase (D-F). **Arrows** indicate period of treatment. In **A** and **B**, vehicle- and IGF-1-treated groups showed significantly different mean clinical scores at several time points. In **A**, using the Student *t*-test, IGF-1 provided protection at day 7 ( $P = 0.024$ ), day 14 ( $P = 0.021$ ), and day 17 ( $P = 0.041$ ) and using the Mann-Whitney sum of rank analysis, on day 10 ( $P = 0.016$ ). In **B**, the scores were significantly different on day 6 ( $P = 0.009$ , Mann-Whitney), and on day 7 ( $P = 0.022$ , *t*-test). Among the chronic phase groups, no significant differences were seen. *y* axis, clinical grade 1-4; *x* axis, days after transfer of cells.

**Table 1.** Pathology of EAE Mice Treated with IGF-1 during the Acute Phase

Period of treatment	Day sampled	Treatment/control	Inflammation	Demyelination	Remyelination	Wallerian degeneration
3-17 dpt	10	Tx	2.4 ± 0.5	1.3 ± 1.1	0	0
		Con	3.7 ± 0.7	2.7 ± 0.7	0	0
	14	Tx	2.7 ± 0.7	1.5 ± 1.2	1.4 ± 0.5	1.0 ± 0
		Con	2.9 ± 0.6	2.5 ± 1.1	1.7 ± 0.5	2.2 ± 0.4
		Tx	1.1 ± 1.0	0.8 ± 1.0	1.0 ± 0	0
		Con	2.7 ± 0.5	2 ± 0.8	2.0 ± 0	1.5 ± 0.5
3-17 dpt	29	Tx	1.8 ± 0.6	1.0 ± 0.8	1.5 ± 0.5	1.8 ± 0.4
		Con	2 ± 0.5	1.1 ± 0.8	1.8 ± 0.4	2.2 ± 0.9
	71	Tx	2.1 ± 0.6	1.9 ± 1.0	1.2 ± 0.2	1.5 ± 0.5
		Con	1.8 ± 0.7	1.3 ± 0.7	0	0
		Tx	0	0	0	0
		Con	2.8 ± 1.7	2.7 ± 2.6	1.0 ± 0	2.0 ± 0
3-17 dpt	11 mo	Tx	1.8 ± 0.3	1.7 ± 0.9	1.0 ± 0	0
		Con	1.6 ± 0.5	1.6 ± 0.7	1.2 ± 0.4	0

treated with IGF-1, although some indication of an influence on immunoregulatory cytokines was seen.

## Materials and Methods

### Animals and Induction of EAE

Female SJL/J mice (Jackson Laboratory, Bar Harbor, ME), were maintained in an NIH/AAALAC-approved facility. Donor mice were immunized at 5 to 12 weeks of age with an emulsion of 0.4 mg/ml bovine myelin basic protein (MBP; Sigma, St. Louis, MO), dissolved in PBS and emulsified with incomplete Freund's adjuvant containing 60 µg of *M. tuberculosis* H37Ra (Difco Labs., Detroit, MI). Ten days later, lymph nodes were removed from these mice, the tissue homogenized into a single cell suspension, and the resultant cells cultured in the presence of 50 µg/ml of MBP for 3 days. These cells were then harvested, washed, counted and injected at a dose of  $3 \times 10^7$  cells/0.2 ml into the tail vein of 5- to 6-week old naïve syngeneic recipients. Onset of signs in recipients occurred 6 to 10 days post-transfer (dpt) of cells. To prevent possible adverse physiological effects (hypoglycemia) in the IGF-1-treated groups, the diets of all animals were supplemented by the addition of sugar cubes to the regular mouse chow. Animals were graded according to a standard clinical index: 1 = limp tail; 2 = hind limb weakness; 3 = one limb plegic; 4 = plegia of 2 limbs; 5 = moribund or dead.<sup>4</sup> A total of 99 mice sensitized for EAE were examined for this study (51 IGF-1-treated, 48 control).

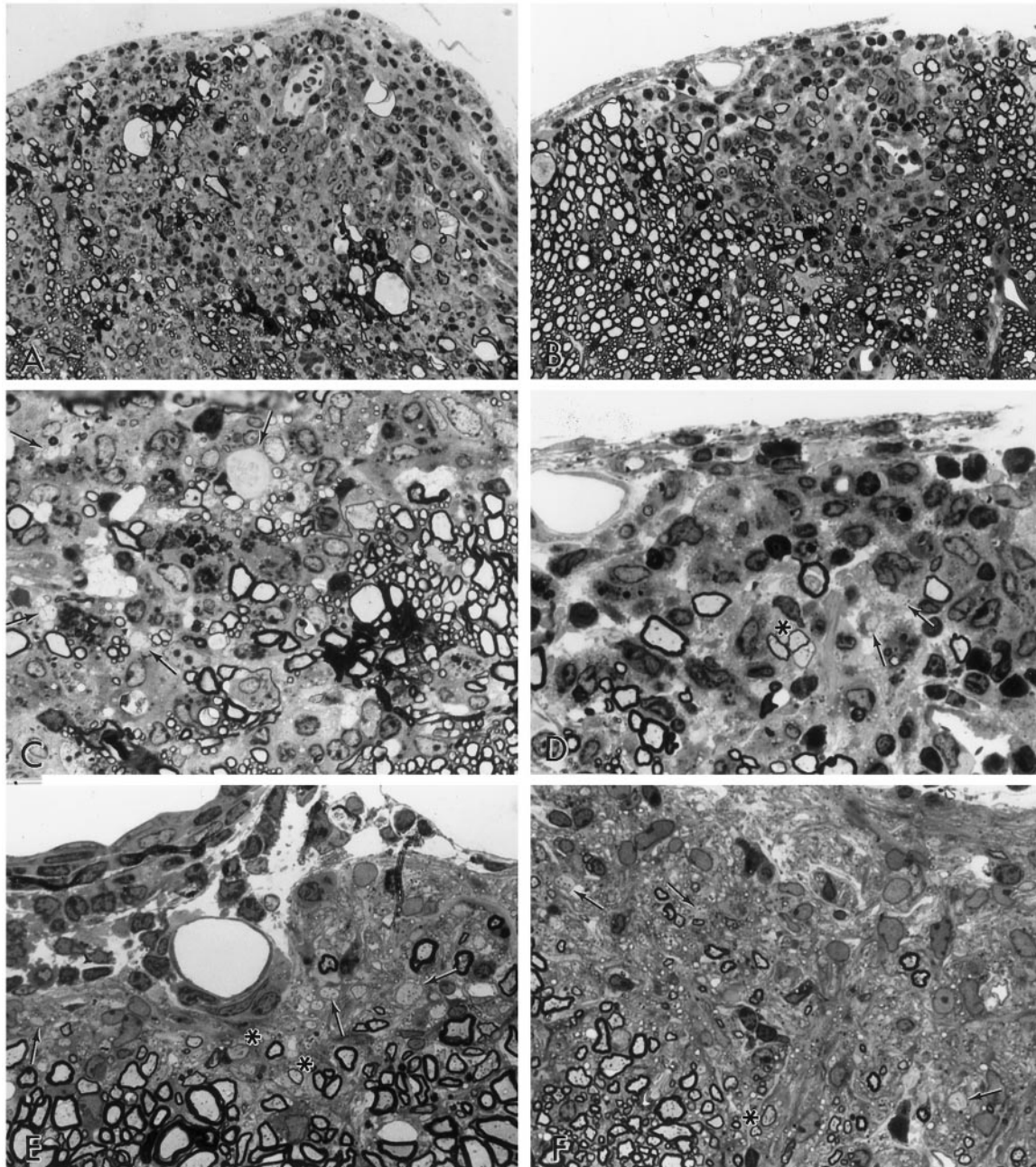
### Treatment with IGF-1

Recombinant human IGF-1 (rhIGF-1) was provided by Genentech (S. San Francisco, CA). The IGF-1 was suspended in a vehicle solution consisting of 10 mmol/L acetic acid, 0.1 mol/L NaCl, and 0.1% Tween, pH 5.4. To determine dose, vehicle and two doses of rhIGF-1 (100 µg and 200 µg) in vehicle were tested using osmotic

pumps implanted under the skin over the animal's back on day 3 post-transfer (pt) of cells and removed 14 days later. Twenty-four animals were tested, 8 in each group, under code. Animals given 100 µg of IGF-1 displayed greater amelioration of EAE than the 200 µg IGF-1 group. Based on the better clinical outcome, a dose of 100 µg of IGF-1 was selected. Animals were treated by subcutaneous (s.c.) injection of the selected dose of IGF-1 or vehicle, delivered twice daily for 14 days before onset of signs (3 to 17 dpt), and at different stages (beginning at 25 to 78 dpt) of the chronic phase of EAE. Control and IGF-1-treated groups were observed clinically and scored under code for effects on disease onset, severity, and relapse rate.

### Neuropathology

For neuropathological analysis, representative animals were sampled at selected time points during and after treatment. Ether-anesthetized mice were perfused with 2.5% glutaraldehyde in phosphate buffer. CNS tissue was removed and thin slices taken from 10 levels of the neuraxis (optic nerve, cerebrum, cerebellum/brainstem, cervical, thoracic, lumbar and sacral cord, and spinal nerve roots). These were postfixed in 1% osmium tetroxide for 1 hour, dehydrated, and embedded in epoxy resin. One-micrometer epoxy sections were stained with toluidine blue and examined by light microscopy in a blinded fashion by two investigators. A score from 0 to 5 was determined for inflammation, demyelination, remyelination, and Wallerian degeneration, based on the degree of CNS involvement, according to established criteria.<sup>3,10</sup> Remyelination was scored as follows: 1 = scattered thinly remyelinated fibers along the lesion margin; 2 = a narrow rim or small groups of remyelinated axons along the lesion margin; 3 = larger groups of remyelinating fibers; 4 = extensive remyelination of lesions; 5 = total remyelination. For electron microscopy (EM), thin sections were contrasted with uranyl acetate and lead citrate and examined in a Siemens 101.



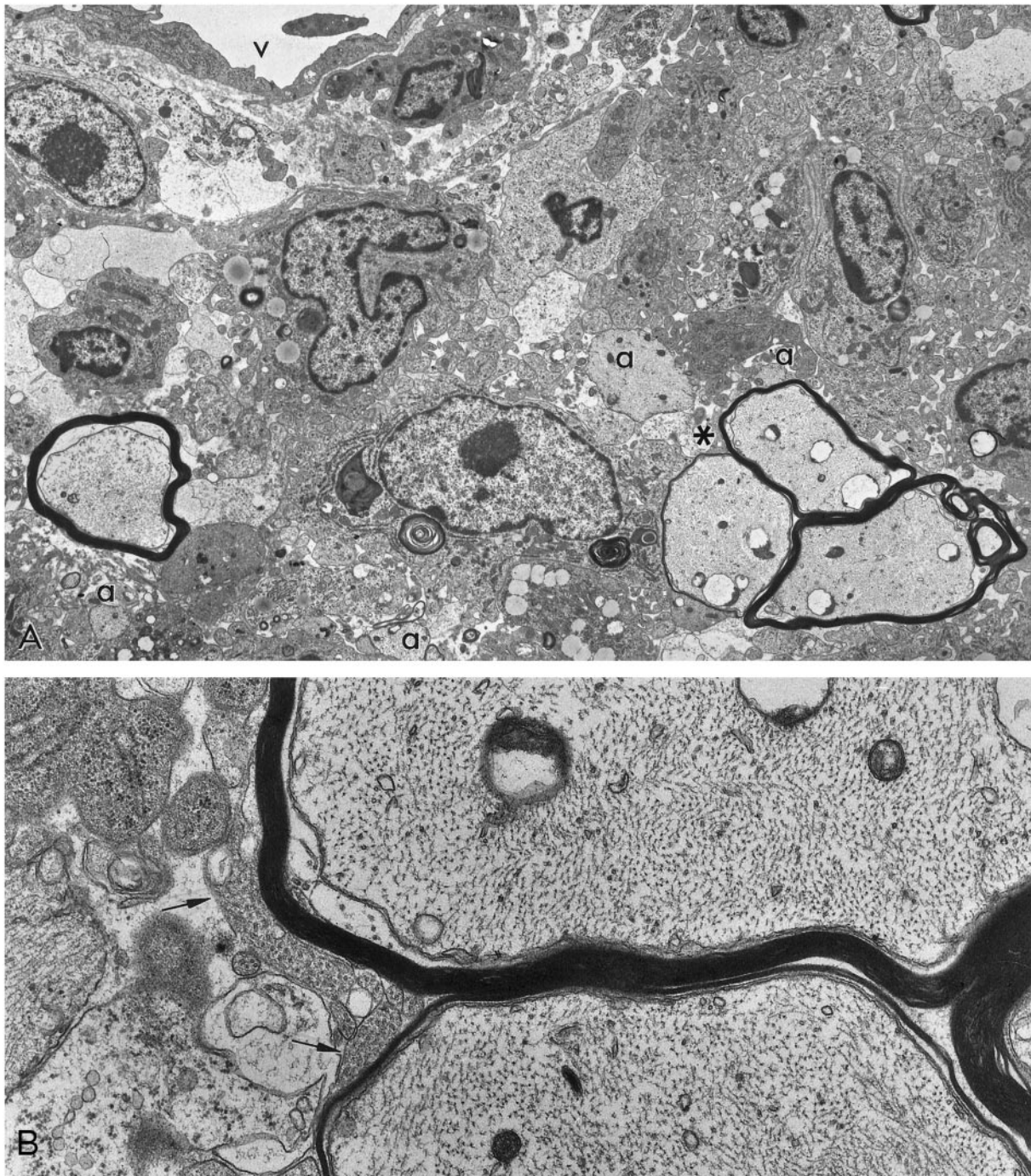
**Figure 2.** Pathology of lumbar spinal cord tissue from animals treated during the acute phase with IGF-1 and vehicle, sampled day 10 pt (**A–D**), and after 11 months pt (**E–F**). One-micrometer epoxy sections stained with toluidine blue. **A:** Vehicle-treated, 10 dpt, clinical score, grade 4. A large inflammatory demyelinated lesion is seen in an anterior column ( $\times 300$ ). **B:** IGF-1-treated, 10 dpt, grade 1. Matching treated group for **A**. A less extensive inflammatory demyelinating lesion is seen in the anterior column. The **asterisk** indicates three fibers shown in detail in **D** ( $\times 300$ ). **C:** Detail from **A** to show demyelinated axons (**arrows**) and macrophages containing myelin debris ( $\times 750$ ). **D:** Same lesion as in **B**, higher magnification. The group of three fibers (shown in **B**) possess thin myelin sheaths suggestive of remyelination (**asterisk**). Demyelinated axons are also apparent (**arrows**) ( $\times 750$ ). **E:** Vehicle-treated, 11 months pt, grade 3.5. A discrete zone of chronic demyelination and glial scarring in the subpial layer is seen. Note the glial scarring and demyelinated axons (**arrows**). A few remyelinated axons are present (**asterisks**) ( $\times 750$ ). **F:** IGF-1-treated, 11 months pt, grade 2. In a matching animal, a lesion comparable in texture to **E** is seen. Demyelinated axons are shown at the **arrows** ( $\times 750$ ).

### Immunocytochemistry

Animals were perfused with PBS, the CNS removed, and slices from cerebral hemispheres, cerebellum, cervical, thoracic and lumbar cord embedded in OCT in a dry-ice bath. Frozen sections were fixed in acetone for 10 minutes and stained using the avidin-biotin-peroxidase com-

plex technique (Vector Laboratories, Burlingame, CA). Overnight incubations were performed at 4°C with the following primary antibodies: rabbit anti-human IGF-1R (Santa Cruz Biotechnology Inc., Santa Cruz, CA) at 1:100 dilution; mouse anti-human IGF-1R (PharMingen, San Diego, CA) at 1:20; rabbit anti-human PDGF-R7 (a kind gift of C-H. Heldin of the Ludwig Institute for Cancer Re-





**Figure 3. A:** EM of a CNS lesion is shown from a mouse with EAE treated with IGF-1 during the acute phase and sampled day 10 pt (same field as shown in Figure 2, B and D). The group of three thinly myelinated axons (**asterisk**) is seen to lie in the midst of a demyelinated, inflamed lesion. Vessel (V) above, demyelinated axons (a) ( $\times 3750$ ). **B:** Detail of **A** illustrating the disproportionately thin myelin sheaths and oligodendroglial cytoplasmic tongues (**arrows**), features usually associated with ongoing myelination and remyelination ( $\times 16,000$ ).

search, Uppsala, Sweden) at 1:200; rabbit anti-rat NG2 (a kind gift of W. B. Stallcup, La Jolla, CA) at 1:100; anti-CNPase (Sigma) at 1:20; and rabbit anti-mouse MBP exon-2 (a kind gift of Dr. Carol Readhead, University of Southern California). Slides were scored in a blinded fashion by two observers on a scale of 0 to 4 based on the density of labeled cells.

### *Ribonuclease Protection Assay*

For RNA preparation, anesthetized mice were perfused transcardially with ice-cold PBS and the spinal cord removed and snap-frozen in liquid nitrogen. Total RNA was extracted with tri-reagent (Molecular Research Center, Cincinnati, OH), as per manufacturer's instructions using

**Table 2.** Pathology of EAE Mice Treated with IGF-1 during the Chronic Phase

Period of treatment	Day sampled	Treatment/control	Inflammation	Demyelination	Remyelination	Wallerian degeneration
25–39 dpt	38	Tx	1.7 ± 0.8	1.3 ± 1.0	0	0
		Con	2.6 ± 0.7	1.2 ± 1.0	0	0
	95	Tx	1.3 ± 0.5	1.1 ± 0.6	1.3 ± 0.5	0
		Con	2.2 ± 0.8	1.6 ± 0.2	1.8 ± 0.4	2.0 ± 0.9
30–44 dpt	53	Tx	2.5 ± 1.8	1.1 ± 0.9	2.0 ± 1	2.3 ± 0.5
		Con	2.0 ± 0.7	1.1 ± 0.8	1.4 ± 0.5	1.7 ± 0.4
41–55 dpt	60	Tx	2.1 ± 0.7	1.9 ± 0.7	1.3 ± 0.5	2.6 ± 0.5
		Con	2.2 ± 0.4	2.2 ± 1.2	1.3 ± 0.4	2.7 ± 0.7
65–79 dpt	93	Tx	2.6 ± 0.7	2.3 ± 0.5	1.8 ± 0.4	2.5 ± 0.5
		Con	2.2 ± 0.6	1.9 ± 0.9	1.3 ± 0.5	2.0 ± 0
78–92 dpt	171	Tx	2.2 ± 1.5	1.3 ± 1.0	1.7 ± 0.5	0
		Con	1.8 ± 0.8	1.7 ± 0.9	0	0

a Power Gen 125 homogenizer (Fisher Scientific), and adjusted to 5 µg/µl RNase-free H<sub>2</sub>O. The expression of various cytokine mRNAs was determined using a multi-probe RPA template set (Riboquant; PharMingen). A total of 20 µg total RNA from spinal cord was hybridized overnight to the [<sup>32</sup>P]UTP-labeled cRNA transcripts of the mCK-3b probe set using the RPA II Kit (Ambion, Austin, TX). Non-hybridized RNA was digested and the protected fragments were precipitated, dissolved in loading buffer and loaded on a denaturing 5% acrylamide gel. Bands were detected by autoradiography and quantified by phosphoimaging on a Storm 860 scanner using the ImageQuaNT 3.01 software package (Molecular Dynamics, San Francisco, CA). Results were calculated as a ratio of the volume of the band of interest to the sum of the bands for the housekeeping genes, L32 and GAPDH. Statistical significance was determined by Student's *t*-test and ANOVA with a threshold value of *P* < 0.01.

### Statistical Methods

The effect of treatment on clinical outcome was assessed by Student's *t*-test and when the data were not normally distributed, the Mann-Whitney sum of ranks test.

## Results

### Clinical Findings

The clinical charts of six representative experimental groups are shown in Figure 1. There were no significant differences in body weight between treated and control groups.

#### IGF-1 Treatment during the Acute Phase

Mice treated with rhIGF-1 preceding and during the acute phase of adoptively transferred EAE (2 × 100 µg rhIGF-1 per diem, s.c. for 14 days beginning 3 dpt), displayed a less severe clinical course commencing on the same day of onset as vehicle-treated animals (4–6 dpt) (Figure 1). In one experiment (Figure 1A), the clinical course of the IGF-1-treated group with EAE (*n* = 5), remained at least one clinical grade lower than the vehi-

cle-treated control group (*n* = 5), the differences being significant at day 7 (*P* = 0.024), day 14 (*P* = 0.021), day 17 (*P* = 0.041) using the *t*-test, and on day 10 (*P* = 0.016) using the Mann-Whitney sum of ranks test. However, in the subsequent experiment (Figure 1B), the clinical scores for the two groups were different only at day 6 (*P* = 0.009, Mann-Whitney) and day 7 (*P* = 0.022, *t*-test). In the third experiment (Figure 1C), there were no differences.

#### IGF-1 Treatment during the Chronic Phase

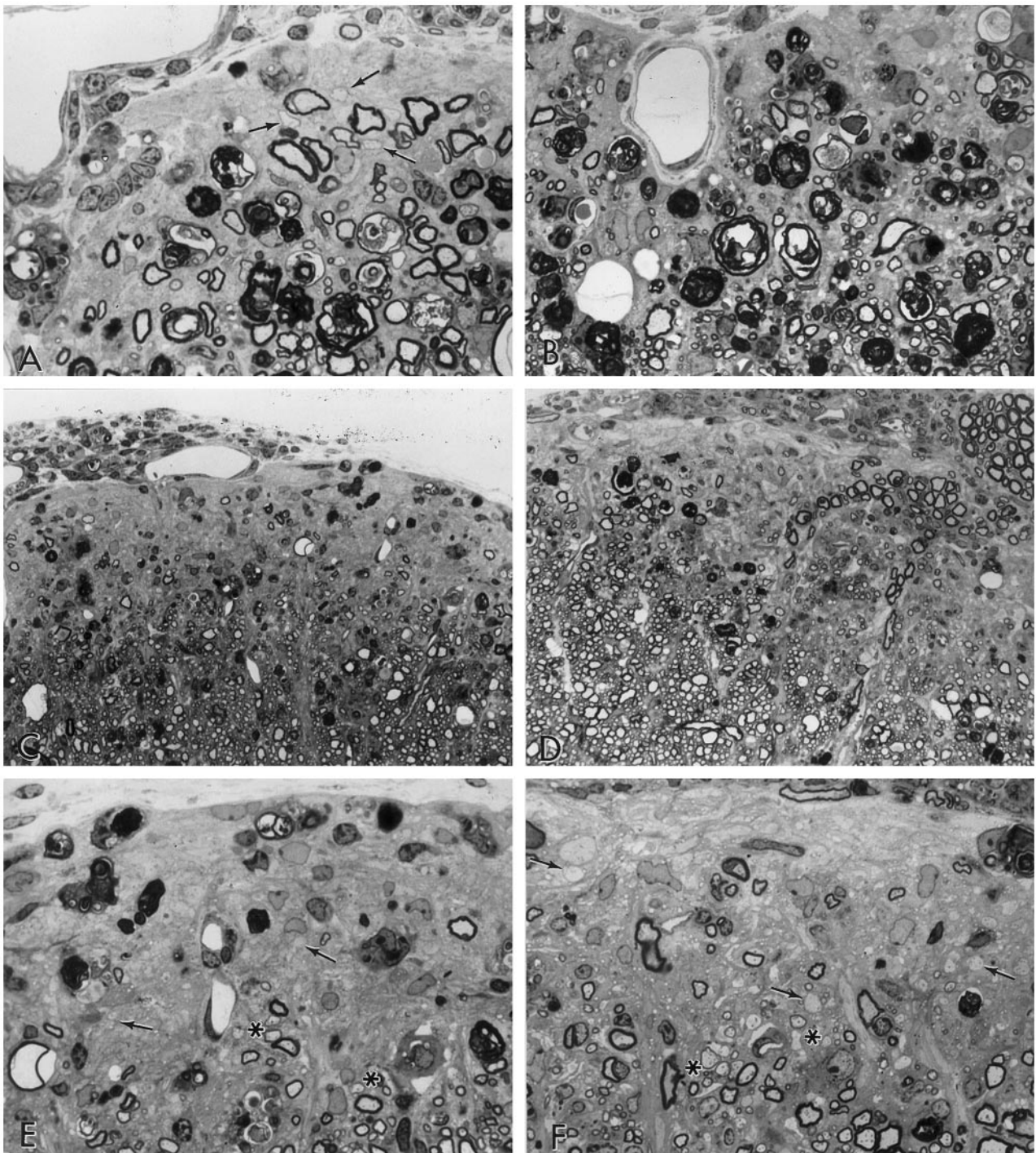
Three experiments were conducted during which mice with established chronic EAE were administered rhIGF-1 at different time points post-transfer (viz. days 25–39, days 41–55, and days 65–79 pt; Figure 1, D–F). In the first experiment, the clinical course of the IGF-1-treated group (*n* = 5), appeared to improve posttreatment. In the second and third groups, the IGF-1-treated animals (*n* = 3), maintained courses similar to those of the vehicle-treated controls (Figure 1, D–F).

### Neuropathology

#### IGF-1 Treatment during the Acute Phase

At early time points during treatment, CNS lesions in IGF-1-treated animals were less inflammatory and demyelinating than in vehicle-treated controls, an observation correlating with the observed decreased clinical severity (Table 1 and Figure 1). However, with time, as the clinical course of both groups became similar, the histopathology was comparable to the extent that the two groups could not be distinguished. In general, however, control groups tended to show more Wallerian degeneration (axonal pathology), than treated groups during early time points (Table 1). Lesions were typically distributed around the subpial regions of the lumbar spinal cord (Figure 2A). Interestingly, while control animals sampled during the acute phase of disease had inflammatory demyelinating lesions, similar to IGF-1-treated groups, one animal from an IGF-1 group sampled on day 10 pt, displayed suggestions of low level remyelination (Figure 2, B and D), with several fibers possessing disproportionately



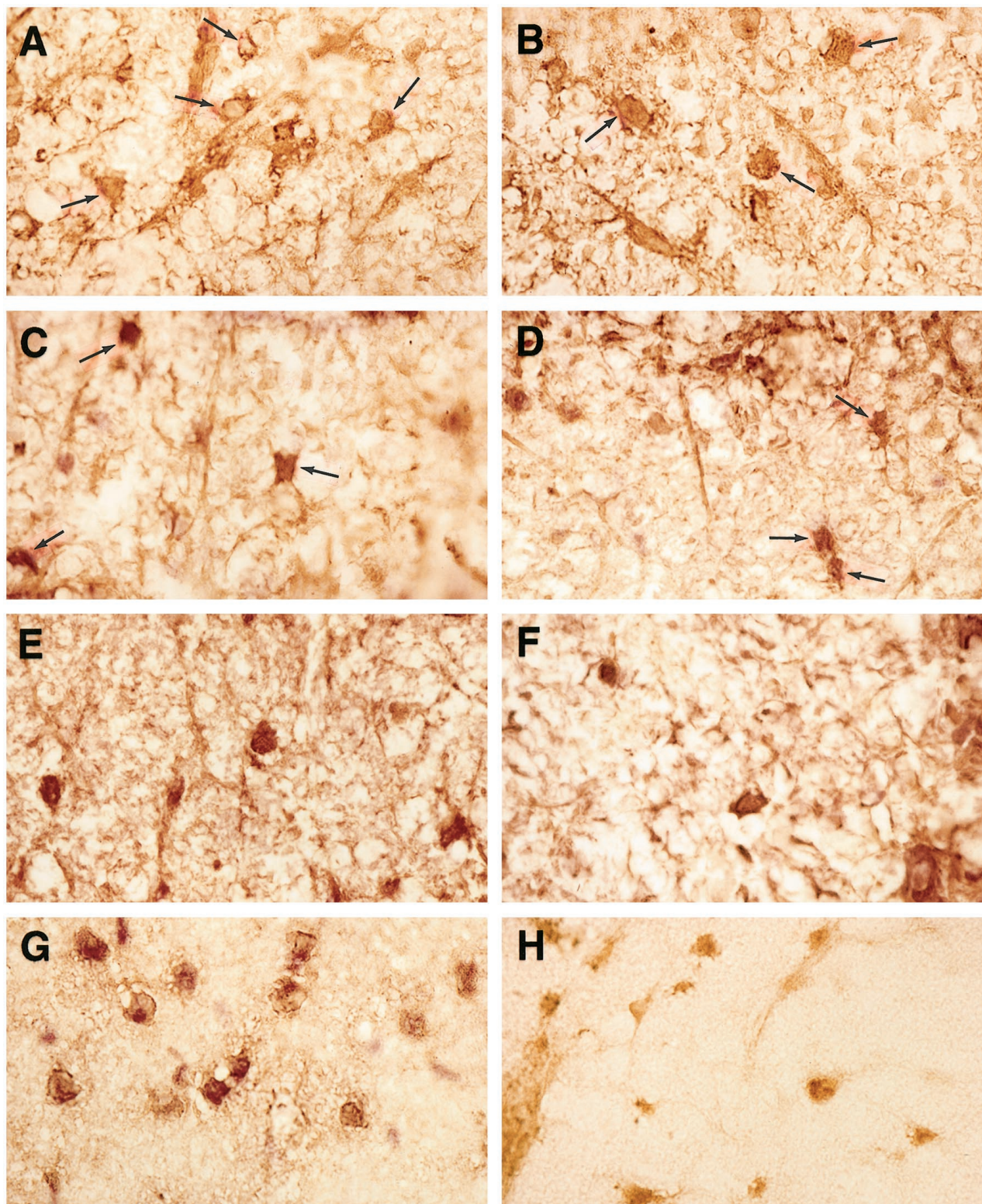


**Figure 4.** Lumbar spinal cord tissue from mice with EAE treated with vehicle and IGF-1 during the chronic phase (41–55 dpt), and sampled day 60 (A and B), and 65–79 dpt, sampled day 93 (C–F). **A:** Vehicle-treated, grade 3. A gliotic demyelinated lesion is seen in the subpial zone. Some Wallerian degeneration and a few remyelinated fibers (arrows) are present. Inflammatory cells are located within the meningeal space above ( $\times 750$ ). **B:** IGF-1-treated, grade 2, matching animal to A. A small lesion lies along the subpial margin. Note the extensive glial scarring, Wallerian degeneration, and a few infiltrating cells above ( $\times 750$ ). **C:** Vehicle-treated, grade 2.5. A large chronically demyelinated lesion lies in an anterior column. Some inflammation is seen in the leptomeninges above ( $\times 300$ ). **D:** IGF-1-treated, grade 3.5; matching animal to C. A lesion similar to the control is shown. Note the inflammation in the leptomeninges, above, and the widespread gliosis ( $\times 300$ ). **E:** Detail of C to show glial scarring, chronically demyelinated axons (arrows) and Wallerian degeneration. Remyelinated axons are seen at the asterisks ( $\times 750$ ). **F:** Detail of D to show scattered chronically demyelinated axons (arrows) and a few remyelinated fibers (asterisks) ( $\times 750$ ).

thin myelin sheaths around large diameter axons. These fibers existed in areas showing ongoing inflammation and demyelination. These same areas were examined by EM and remyelination was confirmed. The thin myelin

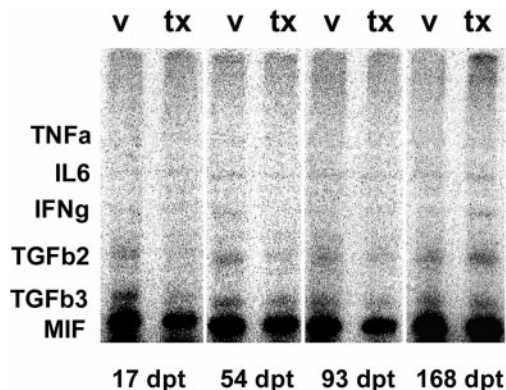
sheaths were associated with numerous outer loops of oligodendroglial cytoplasm, (Figure 3,A and B), features reminiscent of early myelination.<sup>11</sup> Control-treated and animals treated with IGF-1 during the acute phase and





**Figure 5.** Lumbar spinal cord tissue from mice with EAE treated with IGF-1 or vehicle, immunoreacted for oligodendrocyte progenitors. No differences were observed between IGF-1 and control-treated groups at any time point. **A:** IGF-1 treatment during acute phase, sampled day 17 pt. Immunoreacted for IGF-1R. Several small rounded oligodendrocytes (**arrows**) show cytoplasmic staining ( $\times 750$ ). **B:** Same animal as **A**, immunoreacted for PDGF-R7, a marker for oligodendrocyte progenitors. Several oligodendrocytes (**arrows**) display positive staining ( $\times 750$ ). **C:** IGF-1 treatment, acute phase, sampled day 71 pt, immunoreacted for exon 2 MBP. Several oligodendrocytes (**arrows**) are intensely immunoreactive for this marker of immature oligodendrocytes ( $\times 750$ ). **D:** Vehicle treatment, acute phase, sampled day 71 pt. Similar exon 2 MBP immunoreactivity is seen on two oligodendrocytes ( $\times 750$ ). **E:** IGF-1 treatment, chronic phase (25–39 dpt), sampled day 52 pt, exon 2 MBP immunoreacted. A row of positively stained oligodendrocytes is seen ( $\times 750$ ). **F:** Vehicle treatment, matching control for **E**, exon 2 MBP immunoreacted. Two oligodendrocytes stain intensely. Lesion activity is seen to the right ( $\times 750$ ). **G:** Same animal as in **A**, CNPase immunoreacted. Note the intense staining of many oligodendrocytes ( $\times 750$ ). **H:** Same animal as **A**, anti-MAG immunoreacted. Oligodendrocytes display moderate levels of immunoreactivity ( $\times 750$ ).





**Figure 6.** Multiprobe RPA analysis of cytokine mRNA expression in the spinal cord of IGF-1 (tx) and vehicle (v) treated mice measured at selected time points after treatment. The CNS was removed from saline-perfused animals and subjected to RPA analysis using the mCK-3b multiprobe template set (see Methods). Note that expression of TGF- $\beta$ 2 is decreased after treatment at one chronic time point. Also, TGF- $\beta$ 3 shows significant decreases after treatment. Figure 7 illustrates the quantitative analysis of these two cytokines, in addition to TNF- $\alpha$ , IFN- $\gamma$ , IL-6, and MIF, none of which display significant changes.

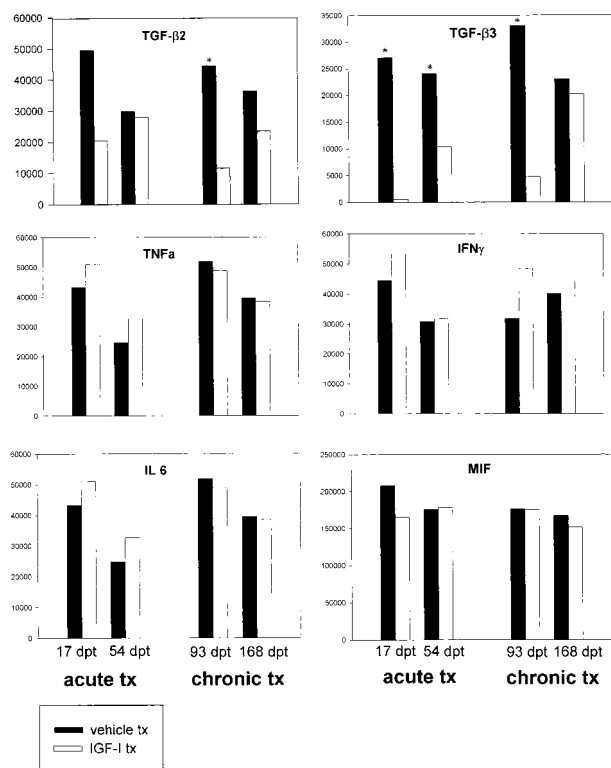
maintained long-term (up to 11 months pt), displayed spinal cord lesions that were indistinguishable from one another, being intensely gliotic with some residual inflammatory activity (Figure 2, E and F). Such lesions contained numerous chronically demyelinated axons and a

few remyelinated fibers toward the periphery. However, there was no difference in the degree of remyelination in both groups.

#### IGF-1 Treatment during the Chronic Phase

In all, five experiments were conducted in which mice that had developed chronic EAE were treated with IGF-1 and compared with vehicle-treated controls (Table 2). Except for a reduced amount of inflammation in one group, IGF-1-injected animals treated from 25 to 39 dpt, the same group showing a clinical difference (Figure 1D), there were no differences in any of the pathological parameters examined (inflammation, demyelination, remyelination and Wallerian degeneration) (Table 2). Variation in pathology was noted in groups treated at different time points post-transfer, although there were no apparent effects attributable to IGF-1. For example, in one group given IGF-1 from 41 to 55 dpt and examined on day 60, both groups still displayed abundant inflammation and Wallerian degeneration while the levels of demyelination and remyelination were unremarkable (Figure 4, A and B). In another group treated from 65–79 dpt and sampled on day 93, more typical gliotic and demyelinated lesions were seen (Figure 4, C–F). None of the mice treated with IGF-1 during the chronic phase of EAE displayed a level of CNS remyelination significantly different from vehicle-treated controls.

#### Cytokine mRNA expression in the spinal cord of IGF-1- and control treated mice



**Figure 7.** The gel shown in Figure 6 was phosphorimaged and the volumes of the protracted bands normalized to the bands for L32 and GAPDH. The data shown for TGF- $\beta$ 3 illustrates statistically significant differences in relative levels of mRNA between the vehicle- and IGF-1-treated animals at both time points, as is also the case for TGF- $\beta$ 2 at day 93 pt (**asterisks**).

#### Immunocytochemistry for Early Oligodendrocyte Markers

As part of our study on the possible effects of IGF-1 on remyelination in this model, frozen sections immunoreacted for IGF-1R, PDGF-R, and MBP exon 2 transcripts were examined from all treated and control groups at different time points post transfer. In most cases, IGF-1R staining was seen on oligodendrocytes although the level of expression was low and similar between groups (Figure 5A). Occasionally, astrocytes displayed immunoreactivity for IGF-1R. Staining for PDGF-R, a marker for progenitor oligodendrocytes,<sup>12–14</sup> was obtained in sections from both acute and chronic IGF-1-treated and control groups where scattered oligodendrocytes throughout the white matter displayed immunoreactivity (Figure 5B). There was some increase in immunoreactivity for PDGF-R toward the margins of lesions but there were no differences in pattern at any time point between IGF-1 and control-treated mice. With a polyclonal rabbit antibody raised against mouse MBP exon 2 peptide, evidence for immature or remyelinating oligodendrocytes,<sup>15</sup> was sought in both IGF-1-treated and control mice, at early and late time points. In all animals, a few MBP exon 2-positive oligodendrocytes could be found at the margins of CNS lesions and scattered throughout the spinal cord white matter (Figure 5, C–F). The numbers of MBP exon 2 positive cells were similar in IGF-1 and control-treated mice. Oligodendrocyte phenotype was confirmed with parallel sections stained for CNPase and MAG (Figure 4, G and H).

### RPA Assay

Data obtained with the mCK-3b set for cytokines from spinal cord mRNA from IGF-1-treated and control mice are shown (Figure 6). No differences were noted for the expression of mRNA for TNF- $\alpha$ , IFN- $\gamma$ , IL-6, and MIF. LT and TGF- $\beta$ 1 levels were not detectable in any of the samples. However, significant differences in the relative levels of mRNA were observed for TGF- $\beta$ 2 on day 93 pt of the chronic treatment group, and for TGF- $\beta$ 3 at both time points during acute treatment and the first time point in the chronic treatment groups (Figure 7). The levels of these two cytokines were much reduced in IGF-1-treated mice.

### Discussion

Based on the well known affinity of the growth factor, IGF-1, to promote proliferation and differentiation of oligodendrocytes *in vitro*,<sup>5,6</sup> and the promising previously demonstrated beneficial effects of this growth factor on the course of actively induced EAE,<sup>7,16</sup> the present study was initiated to test whether prolonged treatment at different stages of adoptively transferred EAE in SJL mice might influence myelin repair. The findings have shown that administration of IGF-1 to animals with EAE, during both the acute and chronic phases, had both positive and negative effects on disease outcome; positive, in that treatment before clinical onset clearly delayed and decreased both clinical signs and lesion formation, albeit transiently; and negative, in that clinical course and lesion histopathology eventually became indistinguishable between IGF-1 and control-treated animals. These findings are in agreement with a more recent study from another laboratory,<sup>9</sup> which showed that during treatment of acute EAE, IGF-1 was capable of reducing the clinical severity and the intensity of CNS inflammation, thus suggesting an effect on T cells, perhaps via IGF-1 receptors known to be expressed on these cells.<sup>17,18</sup> However, as stated above, in the present experiments, this increased efficacy was transient and the clinical and histopathological outcome in all groups ultimately became equivalent. It was demonstrated in a (PL $\times$ SJL/J) F1 mouse model of adoptive EAE<sup>9</sup> that a similar dose of IGF-1 had a mild protective effect when delivered in mice with acute EAE but, once disease developed, no significant effect. On the other hand, another laboratory showed that lower doses of IGF-1, resulted in decreases in relapse rate, lesion burden and inflammation after both acute and chronic treatment of SJL mice with adoptive EAE. However, enhanced remyelination, an anticipated finding based on earlier studies on EAE in the rat by the same group where IGF-1 treatment enhanced myelin protein synthesis,<sup>16</sup> was not found. The present study, which used more sensitive fine structural and immunocytochemical techniques for oligodendrocyte progenitors, was also unable to document any effect of IGF-1 on myelin regeneration.

That a previous<sup>9</sup> and the present study on the IGF-1 paradigm in EAE has demonstrated transient beneficial effects suggests a common mechanistic pathway. Previ-

ous workers<sup>9</sup> examined brain and spinal cord from experimental and control groups for adhesion molecule expression and found decreased ICAM-1 in IGF-1-treated mice. This, they suggested, may merely have reflected differences in inflammatory cell infiltration. On the other hand, it may have correlated with the delayed entry of cells into the CNS. Results from the same authors, using lymph node cells stimulated *in vitro* with MBP and/or IGF-1, revealed no significant differences in cytokine profile. From the present work, two cytokines displayed significant differences in expression in the CNS between experimental and control groups. These were TGF- $\beta$ 2 and TGF- $\beta$ 3 and the decreases in expression in the IGF-1-treated groups occurred at both acute and chronic time points. Whether this represents evidence for decreased immunomodulatory cytokine activity (at the mRNA level, at least), has relevance to the transient nature of the beneficial effect on disease severity, remains a possibility in need of further investigation. This is particularly apropos since TGF- $\beta$  may play an important role in oligodendrocyte differentiation,<sup>19</sup> and is an effective therapeutic molecule in the treatment of acute and chronic relapsing EAE.<sup>20-22</sup> More recently, TGF- $\beta$ 2 has been reported to reduce demyelination in the Theiler's virus model,<sup>23</sup> further testifying to its beneficial effect on CNS myelin. These observations may be relevant to the present findings in that the decrease in TGF- $\beta$ 2 and - $\beta$ 3 may be the indirect effect of IGF-1 acting on cells of the immune system and the CNS, where astrocytes have been demonstrated to synthesize TGF- $\beta$ 1, - $\beta$ 2, and - $\beta$ 3.<sup>24</sup>

The observation of "precocious" remyelination seen after treatment during the acute phase of EAE may be genuine and related to a stimulatory effect on oligodendrocytes by IGF-1, as has been shown at the level of myelin gene expression in previous work on rat EAE.<sup>16</sup> However, it was encountered in a single animal only and may have been the result of a number of pleiotropic factors, as has been speculated in acute fulminant MS lesions where remyelination is also a feature,<sup>25,26</sup> the result perhaps of soluble mediators produced by macrophages. Nevertheless, the appearances were consistent with remyelination (as opposed to incomplete demyelination), since it was associated with elaboration of multiple oligodendrocyte processes. On the down side, this appeared to be a transient repair phenomenon, as is believed to be the case in MS. Gene expression by oligodendrocytes examined from the standpoint of progenitor cells and myelin demonstrated a lack of increased involvement of myelinating cells in IGF-1-treated mice.

Taken in concert, under the conditions studied, IGF-1 had a marked transient beneficial effect on the early course of EAE in the mouse when administered before clinical onset, but no measurable clinical or histopathological effect, particularly at the level of remyelination, when delivered during chronic disease. Since the latter situation is that which parallels most closely the course of human MS, it is therefore doubtful that IGF-1 would be efficacious in the human disease. Thus, while manipulation of immune-mediated demyelinating disease with growth factors with known propensities to cause oligodendrocyte stimulation or proliferation provides a novel



approach which avoids complications arising during immune-mediated therapy, careful screening of each growth factor in different models remains essential.

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