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Plasmapheresis Combined With Interferon: An Effective Therapy for Multiple Sclerosis

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The rationale for the use of interferon (IFN) in the treatment of multiple sclerosis (MS) is based on its recognized antiviral and immunomodulating actions. The pathogenesis of MS is believed to be due to an immunologic response in a genetically predisposed individual, localized within the central nervous system white matter, and triggered by exposure to an environmental agent such as a virus. Based on our personal experience we find that the efficacy of IFN therapy is hampered in MS patients by the presence of an interferon inhibitor factor (IIF) in the patients' sera which we have isolated and characterized. When plasmapheresis (PP) was done on 24 MS patients with intermittent 3-day administration of IFN-α and human leukocyte IFN, marked increase of IFN in 18 patients and modest increase in three patients correlated with clinical improvement. Three clinical nonresponders showed no increase in IFN levels following therapy. The ability to remove IIF and lymphokine inhibitor factor (LIF) by PP may explain the successful treatment of our patients. We describe the evaluation of helper T cells, suppressor T cells, HLADR antigen, natural killer cells, and monocyte/macrophage cell populations by flow cytometry before and after PP. A significant increase in these immune-competent cells correlated with marked improvement in Kurtzke disability status scale in 13 patients, while eight stabilized. Patients showing progression of the disease either showed decrease or no change in these parameters after therapy. Encouraging results from this pilot study suggest that PP combined with immunomodulatory regimens of IFN may be an effective therapy for MS.

Key words: immunomodulation, interferon inhibitor factor, human leukocyte IFN

INTRODUCTION

Self-recognition is a critical underpinning of a normally functioning immune system. Antigen-specific T-lymphocyte activation and the role of the major histocompatibility complex (MHC) in this process are only now being understood. The cascade of membrane, cytoplasmic, and nuclear events resulting from that recognition are appreciated in a limited fashion.

Antigen is presented and MHC gene products are recognized simultaneously on antigen-processing cells through a specific T-cell receptor. This dual recognition is necessary to generate a T-cell immune response [1]. Present evidence suggests that self-regulation is accomplished in the manner in which processed antigen is bound to MHC gene products [2]. There is, then, both a positive and a negative control of the T-cell receptor in such presentation [3,4].

Antigen can be presented to CD8 suppressor T-lymphocytes by most cell types in association with MHC class I gene products. Many of these cells so activated are then directly cytotoxic. While antibody recognizes tertiary surface structures of molecules and require complement activation with K effector cells to achieve cell lysis [5], cytotoxic cells attach to a membrane receptor in a calcium-independent step, releasing proteolytic enzymes to induce cell lysis [6]. This is associated with increased cytokine production.

Lymphocyte activation is cytokine dependent. Protein kinase C, which is also activated [7], initiates cell proliferation and further expression of MHC gene products. Interleukin 2 produced in such activation sustains the response and serves to distribute receptor from cytosol to membrane to permit expansion of the process [8]. Interferon-γ is similarly associated with enhanced expression of MHC class II gene products and acts in synergy with interferon-α [9]. Interferon-α, in contrast, downregulates B-cell antibody synthesis but enhances CD8 cytotoxic cell expression [10]. Protein kinase C production can be bypassed by both growth factors and continued mitogenic stimulus with activation of gene sequences leading to further clonal expansion of involved cells [11]. Octreide can block the process [12].

Finally, with lysis of targeted cells by whatever means, there is often a release of inflammatory mediators (especially arachidonic acid metabolites), which may both trigger complement pathways and activate cytotoxic cells, perpetuating the process. While receptor downregulation to cytokines occurs with continuous exposure, continued

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TABLE I. Multiple Sclerosis Patient's Clinical Characteristics Prior to Therapy

Severity of disease			Sex		
Kurtzke DSS	No. of patients	Average age, years	F	M	Average duration of disease in years
9	1	23	1	0	2
8	3	34	2	1	4
7	4	42	2	2	3
6	2	28	1	1	4
5	1	61	0	1	5
4	4	51	1	3	6
3	4	43	2	2	7
2	4	32	3	1	6
1	1	34	1	0	8
5[a]	24	42[a]	13	11	4.2

[a]Results are total/average for each group.

TABLE II. Multiple Sclerosis Treatment Protocol*

Day 1	IFN-α, 3 mil IU, IM
	Human leukocyte IFN, 2 mil IU, IM
	Immunoglobulin, 100 mg, IV
	Methylprednisolone, 80 mg, IV
	Cyclophosphamide, 100 mg, IV
	Octreide, 200 μg, SC
Day 2	IFN-α, 4.5 mil IU, IM
	Human leukocyte IFN, 4 mil IU, IM
	Immunoglobulin, 200 mg, IV
	Methylprednisolone, 80 mg, IV
	Cyclophosphamide, 100 mg, IV
	Octreide, 200 μg, SC
Day 3	IFN-α, 7 mil IU, IM
	Human leukocyte IFN, 6 mil IU, IM
	Immunoglobulin, 200 mg, IV
	Methylprednisolone, 80 mg, IV
	Cyclophosphamide, 100 mg, IV
	Octreide, 200 μg, SC

*Given every 28 days for 4 months.

organ damage may result from perpetuation of the process and recruitment of other responsive cells.

In MS, the offending antigen appears related to myelin basic protein [13]. Of interest is the recognition that interleukin 1–related cytokines such as lymphotoxin are directly toxic to oligodendrogliocytes [14]. Cerebral vessels may represent lymphocyte targets [15]. Coronavirus infection may also enhance antigen expression on oligodendrogliocytes and astrocytes [16]. Post-transcriptional processing of mRNA may also alter antigen expression [17]. Similar mechanisms have also been described in other autoimmune disorders, including myasthenia gravis [18]. Viral persistence may be explained by lack of MHC expression [19].

Therapeutic plasmapheresis (PP) is an effective means for removing a host of humoral factors from the circulation [20]. PP as a therapy for MS has been utilized since it was first proposed a decade ago [21]. The rationale for our treating MS patients with PP is based on the assumptions that MS is an autoimmune disorder and that PP is an effective means of removing antibodies and other proteins from the circulation [22].

Thus, based on our current knowledge of the immunopathogenesis in MS, we have designed an immunomodulatory approach to bring about clinical improvement in patients with progressive MS who were unresponsive to prior conventional therapies.

PATIENTS AND METHODS

Patient Selection

A total of 24 patients (13 female and 11 male, ages ranging 23–61 years) with clinically definite MS [23], who failed previous therapy, gave informed consent to participate in the study. All had a chronically progressive course (continuous worsening in strength, visual changes, or ataxia) for at least 24 months on serial neurological examination (Table I).

Clinical Evaluation

Initial and follow-up evaluations were carried out by a neurologist using Kurtzke disability status scale (KDSS).

Neuro Diagnostic Tests

Magnetic resonance imaging (MRI) was performed in all patients, and plaques typical for MS were identified and confirmed by the radiologist and neurologist. To reduce the cost, this test was not repeated in all responders but was regularly repeated in patients who progressed.

Plasmapheresis Protocol

All patients were hospitalized at the beginning of the treatment, which was initiated after necessary testing. All patients received drug therapy as summarized in Table II. During each PP, 60 ml/kg body weight of patient's plasma was exchanged for 3.5% albumin in normal saline, containing 6.9 mEq Ca^{2+}/L, 1.2 mEq Mg^{2+}/L, and 4 mEq K^{+}/L. Plasma was separated by continuous flow membrane filtration device (COBE) at a rate of 40 to 100 ml/min [24]. PP was done for 2 consecutive days and repeated every 28 days × 4. Interferon (IFN) and interferon inhibitor factor (IIF) levels were checked before and after each PP.

Interferon Protocol

A 3-day PULSE therapy schedule of IFN (using a combination of two types of IFNs) combined with octreide was initiated based on clinical studies [25,26]. The two types of IFN were recombinant IFN-α and human leukocyte IFN (natural production, containing 18 types of IFN). Systemic administration clearly exerts effects within the central nervous system [27]. The average duration of treatments that these patients received was for 2 years except for those who showed stabilization/progression.

Other Modulatory Drugs

Following PP, the patient received low-dose cyclophosphamide, 100 mg IV × 3 days; methylprednisolone, 80 mg IV × 3 days; and intravenous immunoglobulin, 100 mg IV (day 1), 200 mg IV (days 2,3). This was also repeated 28 days × 4 months. The average duration of treatments for these patients was 2 years except for those who showed stabilization/progression.

Interferon Assay

Cell line. The human amnion or "WISH" cells were obtained from American Type Culture Collection (ATCC) and grown in Eagle basal medium (GIBCO) with 15% FBS.

Virus. Vesicular stomatitis virus (VSV) was obtained from ATCC. The VSV stock solution was diluted 1,000 times with basal Eagle medium containing 2% FBS.

Standard interferon. Human recombinant IFN-α was supplied by National Institute of Allergy and Infectious diseases. Human recombinant IFN-γ (polyferon 50) was obtained from Dr. Rentschler, Germany. Human fibroblast IFN and human leukocyte IFN was from Virogen, Basel, Switzerland.

Assay. The standard IFN concentration which showed the most cytopathic effect inhibition of 1:1,000 diluted VSV on 3.5×10^5 WISH cells was measured and calculated. To this predetermined concentration of standard IFN and 3.5×10^5 WISH cells/well, we added patient sera collected before PP, at a dilution of 1:10, 1:20, and 1:40 and incubated for about 2 hours. To this, 1:1,000 diluted VSV was added and plates were incubated at 37°C in a 5% CO_2 water-jacketed incubator. The plates were checked periodically under an inverted NIKON TMS microscope until the desired 90% cytopathic effect (CPE) in the viral control and 50% CPE in the reference was achieved. This takes approximately 24–28 hours. The plates were then stained with neutral red dye after which they were eluted and read on a microtiter Bio Rad plate reader at 540 nm. The above has been previously described in detail [28,29]. Results are reported in International Units/ml.

Calculation for IIF

The level of IIF in sera of MS patients was calculated by determining percent inhibition of protection of WISH cells by standard IFNs from the CPE of VSV. Any elevation in CPE indicates the level of IIF present in serum.

Calculation for Lymphokine Inhibitor Factor

Activation of T lymphocyte subsets by interleukin 2 was first measured on FACScan by CD3/HLADR-positive staining. This mixture of PBL and IL-2 was then incubated with the patient sera, and LIF levels were calculated by determining percent inhibition of activated CD3/HLADR cells.

Immunofluorescence Staining of Cells

Isolation of peripheral blood lymphocytes. Mononuclear cells were isolated by Ficoll-hypaque gradient centrifugation, collected aseptically, and washed twice with PBS.

Flow cytometry. Staining and flow cytometry analysis were performed as described [30], and a FACScan (Becton Dickinson serial no. 81326) was used for two-color analysis. Gates for CD4, CD8, HLADR, NK CD3−/CD56+, and CD68 surface markers were set, and cells stained with fluorescence and/or phycoerythrin dyes were measured.

RESULTS

Twenty-four MS patients (13 female and 11 male) who were becoming progressively worse on conventional therapies (methyl prednisolone) were entered into the study. Inhibitor factor to IFN and to lymphokine were demonstrated in the circulation at the time of protocol entry. In these patients diplopia was marked in seven; there was blurred vision in three, upper extremity paralysis in four, lower extremity paralysis in two with paraparesis of lower extremities noted in six others, and difficulty walking was present in the remainder. Six patients had bladder and bowel dysfunction. The severity of the disease was evaluated by Kurtzke DSS. One patient had Kurtzke level 9; three were at 8, four at 7, two at 6, one at 5, four at 4, four at 3, four at 2, and one at 1 KDSS level. The calculated average degree of Kurtzke was 5 with average age 42 years. The average duration of the disease was 4.2 years (between 2 and 8 years) (Table I).

All patients had low serum IFN with high serum IIF levels prior to initiating therapy. Following PP and administration of IFN + methylprednisolone + cyclophosphamide + octreide + immunoglobulin for a period of 4 consecutive months (Table II), the level of detectable IFN increased (six patients, 25–1,100 IU; three patients, 25–380 IU; ten patients, 25–560 IU; two patients, 25–780 IU) in 21 out of 24 patients (Fig. 1). This correlated with improvement in Kurtzke DSS by 2 to 4 steps as well as stabilization. In three patients who continue to worsen, there was no change in serum IFN levels. The percent IIF which was elevated at the time of first PP (data not shown) decreased to a negligible level after the fourth PP (in eight patients) by about 50% in 13 patients, and there was no change in three patients. This change in IIF had significant impact on the patients clinical status. Five patients showed 4-step improvement on Kurtzke DSS, eight patients improved by 2 steps, while eight stabilized.

The duration of the response for five patients with 4-step improvement was between 4 and 8 years; in eight patients showing 2-step improvement it was 3–6 years; and eight patients stabilized for 2–4 years. None of the

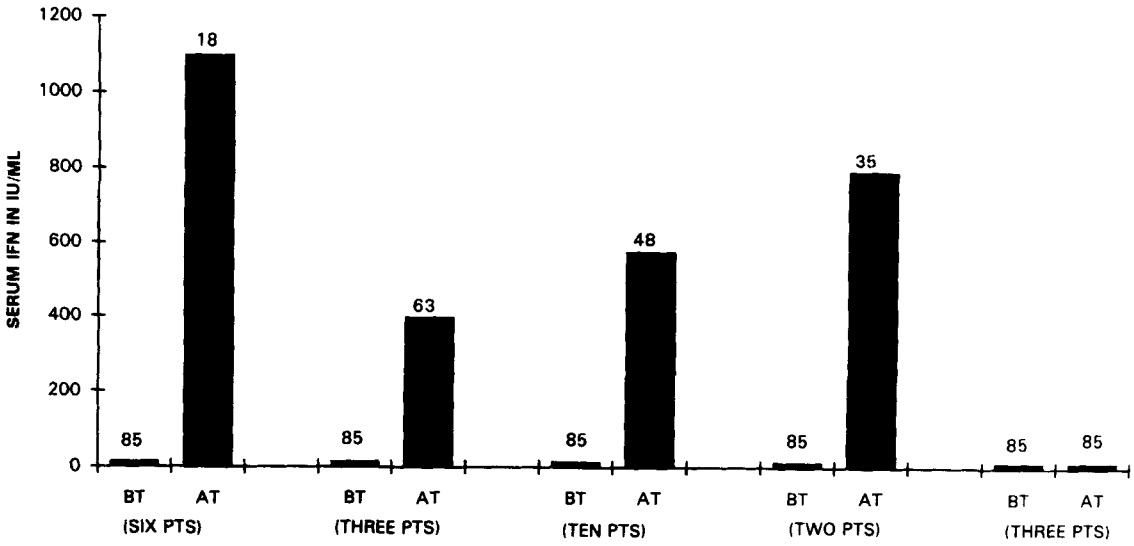

Fig. 1. Elevation in serum IFN level (expressed in IU/ml) after plasmapheresis in MS patients who responded to immunomodulatory therapy. There was no change in three patients who progressed because of disease. Values are IIF levels in percent. BT, before treatment; AT, after treatment.

TABLE III. Immunomodulatory Therapy for Multiple Sclerosis*: Patient Characteristics in Relationship to Response

Response	No. of patients	Kurtzke DDS		Average duration of response to therapy in years	Average age in years	Average duration of disease in years
		Pre TX	Post TX			
Four-step improvement	3	8	4			
	1	7	3	4^+–8^+	32	2
	1	6	2			
Two-step improvement	1	9	7			
	3	7	5	3^+–6^+	36	4
	1	6	4			
	3	4	2			
Stabilization	1	4	4			
	2	3	3	2^+–4^+	48	6
	4	2	2			
	1	1	1			
Progression	1	3	4			
	1	3	5		51	3
	1	5	6			
Total	24	5	3.5			4.2

*Four-category improvement, respiratory and muscle strength; disappearance of tongue and muscle fasciculation; speech improvement. Two-category improvement, reduction of muscle fasciculation; reduction of tongue fasciculation.

patients in this group has relapsed (Table III). No change in number and dimension of MS plaques was observed in MRI. In fact, decrease in dimension of some peracute plaques was observed in some patients, but this probably can be attributed to regression of periplaque edema (data not shown). These patients received four courses of treatments each.

Common to responders was a dramatic fall in circulating IIF and/or lymphokine inhibitor levels (mean fall 70%). Immune stimulation was evident in responders with normalization of circulating immune complexes (67% of patients) and elevations in CD4 counts (46% of patients). CD8, HLADR, NK, serum IFN and monocyte/macrophage cell population increased in responding patients.

Out of five patients who improved 4 steps on Kurtzke, all had <25% CD4+ cells before therapy. The mean percent value of "T" helper cells after therapy changed to 60.5% in two patients, while in the other three patients it became 70.5%. All three patients who progressed due to disease had a "T" helper cell value of 35% or less. Patients showing 4-step improvement on Kurtzke had >31% range of HLADR antigen, while those three patients that progressed all had 15% HLADR antigens. The same pat-

TABLE IV. Immunomodulatory Evaluation of Multiple Sclerosis*

Response	No. of patients	Kurtzke DSS Pre Tx	Kurtzke DSS Post Tx	LIF[a] B Tx	LIF[a] A Tx	IIF[a] B Tx	IIF[a] A Tx	TH[a] B Tx	TH[a] A Tx	TS[a] B Tx	TS[a] A Tx	HLADR[a] B Tx	HLADR[a] A Tx	NK[a] B Tx	NK[a] A Tx	M/M[a] B Tx	M/M[a] A Tx	S-IFN[a] B Tx	S-IFN[a] A Tx
Four-step improvement	3	8	4																
	1	7	3	31	11	27	14	18	32	22	46	11	25	14	28	44	68	5	20
	1	6	2																
Two-step improvement	1	9	7																
	3	7	5	28	18	31	19	19	28	23	33	14	20	18	26	42	52	5	15
	1	6	4																
	3	4	2																
Stabilization	1	4	4																
	2	3	3	27	20	34	30	20	22	24	22	15	15	17	18	40	44	5	6
	4	2	2																
	1	1	1																
Progression	1	3	4																
	1	3	5	29	32	30	34	19	14	22	16	15	14	17	16	41	50	5	5
	1	5	6																
Total	24			115	81	122	97	76	96	91	117	55	74	66	88	167	214	20	46

*Four-category improvement, respiratory and muscle strength; disappearance of tongue and muscle fasciculation; speech improvement. Two-category improvement, reduction of muscle fasciculation; reduction of tongue fasciculation.

[a]Results are average for each group of patients. LIF, lymphokine inhibitor factor; HLADR, cells bearing HLADR antigen; S-IFN, serum interferon level; IIF, interferon inhibitor factor; NK, natural killer cells; B Tx, before therapy; TH, T-helper cells; TS, T-suppressor cells; M/M, monocytes/macrophage; A Tx, after therapy.

tern of results are also seen with the OKT-8 populations and NK cell population (Table IV).

Hypotension during PP was noted in some patients but was readily corrected with hydration. Fever (100–104°F) developed 2 hours after IFN injection, lasted 4–8 hours, was relatively well tolerated, and was the only adverse effect following IFN-α administration.

DISCUSSION

Progress toward an effective treatment for MS has been slow. Because immunologic studies have suggested both a "hyperactive" and a defective immune response in patients with MS [31], experimental treatment regimens designed either to suppress [32] or to enhance [33] the immune response have been attempted. PP in conjunction with immunosuppressive drugs may be an effective therapy in MS [34]. This study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH suggests that the intensive immunosuppression regimen was more effective in halting progression of the disease at both 6 and 12 months. A double-blind controlled study [35] showed PP to be significantly ($P < .007$) effective in delaying the progression of the disease in patients with chronic progressive multiple sclerosis taking immunosuppressive drugs. Also in the Canadian trial [36], at 12, 18, and 24 month assessment, the patients showing stabilization or improvement was greater in the PP group than in other groups. Why PP works in MS is not entirely clear. The rationale for doing PP in MS includes changes in T-cell regulation, presence of immunoglobulin in the cerebrospinal fluid, and a profound lymphocyte infiltrate in the active MS lesion. Our study shows for the first time a correlation between improvement in serum IFN levels following removal of circulating IIF by PP in MS patients. Increased serum IFN levels correlated with clinical improvement.

Both groups of patients, those with 4 KDSS improvement (five patients) and 2 KDSS improvement (eight patients) showed an increase in CD4+ cell population as well as HLADR antigen-bearing cells, thereby indicating an increase in the expression of MHC class II molecules on the macrophage surface. Activation of these molecules results in the release of several cytokines in the circulation.

The immunomodulating agents administered in conjunction with PP were used to alter T lymphocyte expansion (cyclophosphamide) [37], to diminish lymphocyte expansion, cerebrospinal fluid immunoglobulin synthesis, and production of arachidonic acid metabolites (methylprednisolone) [38], and to block Fc-dependent binding of antibody-coated cells (immunoglobulin) [39,40]. Though it is not possible to sort out the effects of these drugs on the course of MS or on the various immunologic studies presented, we feel that PP played a role in the improvement of our MS patients. Further studies are indicated to validate our findings.

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